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Surveillance and Response to Infectious Diseases and Comorbidities:

An African and German Perspective

Proceedings Report
12 – 13 April 2018

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The Academy of Science of South Africa (ASSAf) was inaugurated in May 1996. It was formed in response to the need for an Academy of Science consonant with the dawn of democracy in South Africa: activist in its mission of using science and scholarship for the benefit of society, with a mandate encompassing all scholarly disciplines that use an open-minded and evidence-based approach to build knowledge. ASSAf thus adopted in its name the term 'science' in the singular as reflecting a common way of enquiring rather than an aggregation of different disciplines. Its Members are elected on the basis of a combination of two principal criteria, academic excellence and significant contributions to society.

The Parliament of South Africa passed the Academy of Science of South Africa Act (*No 67 of 2001*), which came into force on 15 May 2002. This made ASSAf the only academy of science in South Africa officially recognised by government and representing the country in the international community of science academies and elsewhere.

This report reflects the proceedings of the *Surveillance and Response to Infectious Diseases and Comorbidities: An African and German Perspective* workshop held from 12 – 13 April 2018 Durban, South Africa, unless otherwise stated.

Views expressed are those of the individuals and not necessarily those of the Academy nor a consensus view of the Academy based on an in-depth evidence-based study.

Surveillance and Response to Infectious Diseases and Comorbidities:

An African and German Perspective

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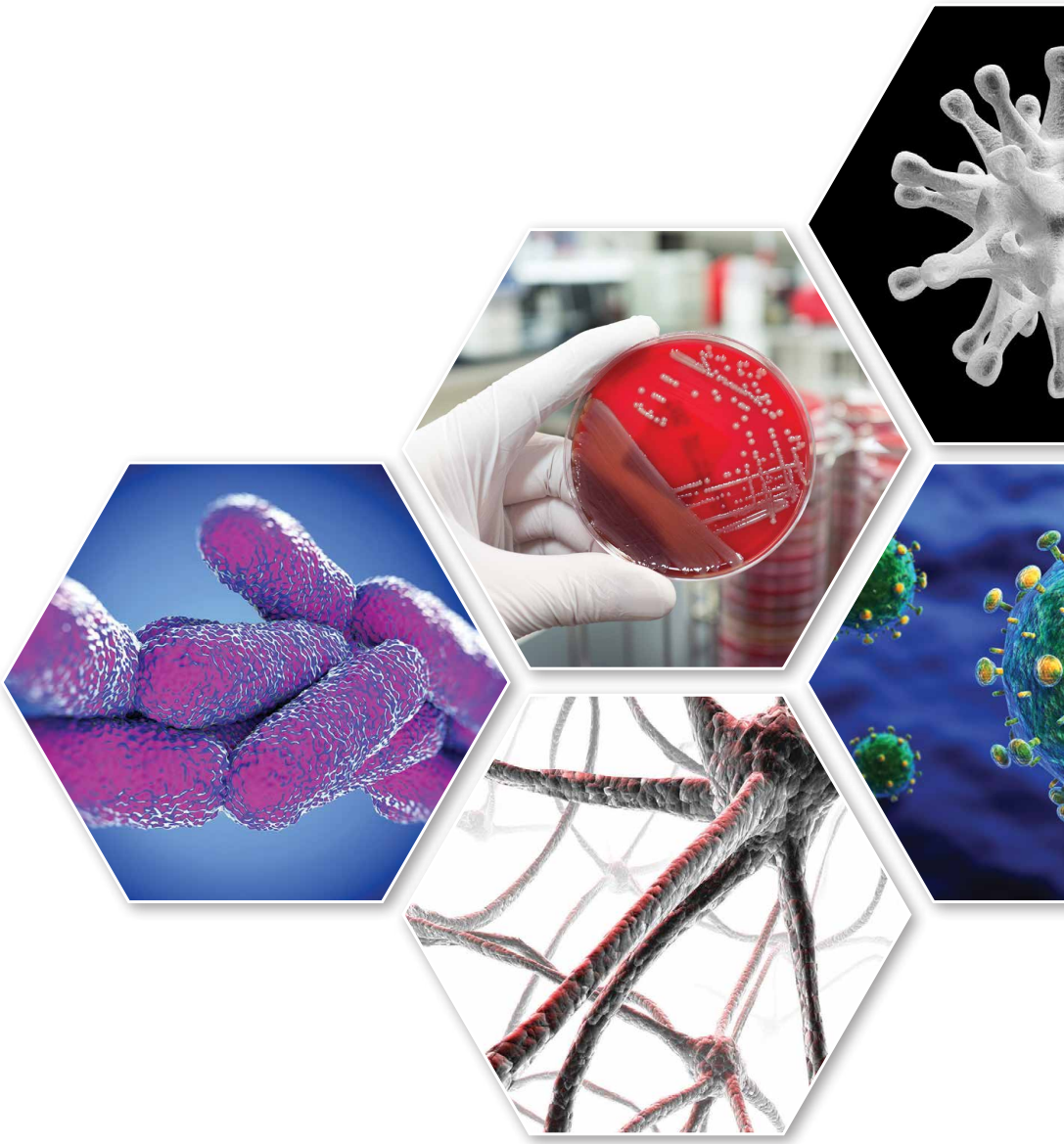

SESSION 1: OPENING

Facilitator: Prof Roseanne Diab, Executive Officer, Academy of Science of South Africa (ASSAf), South Africa

Prof Roseanne Diab opened the conference and welcomed everyone, particularly the delegation from the German National Academy of Sciences Leopoldina (six young scientists, several senior scientists and members of the secretariat) and representatives of the Uganda National Academy of Sciences (UNAS), both of which had collaborated in compiling the programme. UNAS and ASSAf were recipients of an academy development initiative between 2004 and 2011 and had continued their valued relationship. A representative from each of the organising academies had the opportunity to make welcoming remarks.

Welcoming Remarks

Prof Jonathan Jansen, Academy of Science of South Africa, South Africa

Prof Jansen acknowledged the presence of Prof Jörg Hacker, President of Leopoldina; Dr Anban Pillay, Deputy Director-General of the South African Department of Health (DoH); Mr Christian Acemah, Executive Secretary of UNAS; Prof Sabiha Essack, ASSAf Council Member; and Prof Roseanne Diab, Executive Officer of ASSAf. He welcomed everyone to the second Infectious Diseases Symposium jointly hosted by ASSAf, the Leopoldina and UNAS. The first joint symposium on infectious diseases between Leopoldina and ASSAf was held in October 2016 at the Max Planck Institute for Infection Biology in Germany.

The symposium is an opportunity to share knowledge and experience on this difficult topic, which confronts people around the world. ASSAf's relationship with Leopoldina is one of the most productive in the South African science community. He thanked the German colleagues for taking this partnership seriously. A recent event in terms of this partnership was the Science, Business, Society Dialogue Conference hosted in November 2017 in Johannesburg. ASSAf was celebrating five years of cooperation with Leopoldina in 2018. ASSAf was delighted to be working with UNAS and looked forward to a continued relationship.

A vast number of countries in Africa are reported to be ill-equipped in diagnostics, accurate reporting and providing treatment to combat known and emerging infectious diseases. The outbreaks of infectious diseases are expected to persist unless countries have proper diagnostic tools and surveillance responses. The unprecedented epidemic of the Ebola virus in West Africa highlights the need for stronger systems for response and prevention globally. This is a critical conference for identifying and coordinating outbreak action responses nationally and across borders. The sharing of knowledge in this regard is critical. The objective of the conference is to assess the prevalence, surveillance and responses to infectious diseases.

Prof Jansen thanked the scientific coordinators who had compiled the programme for the event: Prof Quarraisha Abdool Karim (ASSAf), Prof Stefan Kaufmann (Leopoldina) and Prof David Serwadda (UNAS).

On behalf of ASSAf, and with gratitude to Leopoldina and UNAS, Prof Jansen welcomed all delegates to the symposium.

Prof Jörg Hacker, President, German National Academy of Sciences Leopoldina, Germany

It is a special pleasure for me to welcome you on behalf of the German National Academy of Sciences Leopoldina to this Inter-Academic Symposium on Surveillance and Response to Infectious Diseases and Comorbidities: An African and German Perspective jointly organised by ASSAf, UNAS and Leopoldina. First, I want to express my thanks to the scientific organisers of the meeting, especially to Prof Quarraisha Abdool Karim for ASSAf, Prof David Serwadda for UNAS and Prof Stefan Kaufmann for Leopoldina. They have taken on an additional load of work to make this conference a reality. Thanks to your commitment, we can look forward to an exciting meeting with brilliant speakers.

Leopoldina is very proud to have a close and vital partnership with ASSAf. In August 2013, the former President of ASSAf, Prof Daya Reddy, and I signed a memorandum of understanding (MoU) in Pretoria in order to strengthen ties between our academies and to provide a permanent platform for future bilateral cooperation.

Within the framework of this cooperation agreement, high-profile joint symposia on topics of substantial scientific, global and social relevance have been organised on a regular basis. Let me just mention the conference on Partial Differential Equations and their Applications in Stellenbosch in 2016 or the conference on Air Pollution and Health in Düsseldorf, Germany in 2017. These events have already filled this MoU with life and promoted scientific exchange between our countries.

Today, President Jansen and I will have the great pleasure of signing an addendum to the existing MoU. I am delighted that this fruitful bilateral cooperation will continue. In the addendum, Leopoldina and ASSAf will stress their willingness to continue their successful cooperation based on the MoU.

The conference today is part of a series that the Leopoldina and ASSAf have established in the area of health research. The series started with a joint conference on Environment and Health in Pretoria in June 2015. During that meeting, the participants analysed key environmental changes and their implications for human health in sub-Saharan Africa and Germany in order to identify future research needs and to assess possible solutions to present environmental health issues. Both academies also cooperated with science academies from Ethiopia and Ghana.

In October 2016, Leopoldina and ASSAf organised the first conference for African and German young scientists in the area of infectious diseases at the Max Planck Institute for Infection Biology in Berlin. The scientific coordinators of the conference were Prof Stefan Kaufmann, Max Planck Institute for Infection Biology, Berlin, Germany on behalf of Leopoldina, and Prof Quarraisha Abdool Karim, University of KwaZulu-Natal, on behalf of ASSAf. This year, I would like to thank both of them for taking over this coordinating task again.

I am most delighted that this conference will be the continuation of the health series. This time, it is a great pleasure for me to also welcome UNAS as an organising partner. I am looking forward to continuing this cooperation with UNAS.

The theme of the conference is Surveillance and Response to Infectious Diseases and Comorbidities: An African and German Perspective. In this

regard, the speakers of the conference will present research results in the areas of antimicrobial resistance (AMR), One Health, comorbidities, human immunodeficiency virus (HIV), tuberculosis (TB), malaria and hepatitis.

Over the past several years, the global emergence of infectious disease outbreaks has revealed that new approaches to fight and prevent the spread of pathogens are urgently needed. Many factors and a combination of factors contribute to infectious disease emergence. Worldwide population growth and urban migration, increasing international travel and trade and globalised food distribution favour the spread of infectious diseases. Mutations, gene transfer and recombination are responsible for pathogen variability, and drug-resistant infections are on the rise. I am pleased that the conference will bring together representatives from a broad range of disciplines to discuss these issues.

It is a special characteristic of this interdisciplinary conference that most speakers are young scientists from Germany and sub-Saharan Africa. ASSAf generously provided scholarships for numerous young scientists from South Africa and other African countries to attend. This variety of African and German speakers makes the current event exceptional. I would like to stress that the promotion of young scientists is a very relevant task of academies in the research area of human health and beyond.

The current conference also promotes the discourse between young scientists and renowned senior scientists. On the German side, I am delighted that apart from Prof Stefan Kaufmann, also Prof Thomas Mettenleiter (President of the Federal Research Institute for Animal Health) and Prof Axel Brakhage (Director of the Leibniz Institute for Natural Product Research and Infection Biology) are attending this conference. These scientists are also members of Leopoldina.

The academies jointly organised a one-day workshop on Science Advice for the young scientists attending this conference yesterday. This workshop was organised in cooperation with the International Network for Governmental Science Advice – Africa (INGSA-Africa) and the International Council of Science Regional Office for Africa (ICSU ROA). The objectives were to strengthen the capacity of young scientists in providing science advice to national governments, as well as to develop and share science advice principles and guidelines pertaining to infectious diseases. I would like to

thank my African colleagues for this initiative, because in future years our academies will need the expertise of young scientists in the frame of their policy advice activities.

My special thanks go to Prof Jonathan Jansen and ASSAf for hosting this conference and their great hospitality. We feel very comfortable in Durban. Moreover, I would like to thank all speakers as well as, again, the organisers of the conference for their great efforts. Finally, I would like to thank Dr Siyavuya Bulani from ASSAf for the excellent organisational management of the conference.

I wish us a successful conference as well as fruitful discussions today and tomorrow.

Mr Christian Acemah, Executive Secretary, Uganda National Academy of Sciences, Uganda

The year 2016 was significant. In February 2016, the first Science Advice workshop took place and has become an important and significant initiative for the African continent, as shown by the event the previous day. In November 2016, UNAS and ASSAf signed an MoU without knowing that the present workshop would take place under the auspices of that agreement. Mr Acemah hoped that the present event signalled a growing relationship with ASSAf. The two academies were in discussions to launch several work-stream studies.

Times were changing. It used to be rare to find two African academies working together in a spirit of mutual respect and accountability, but this was happening more and more on different sides of the continent and on various issues. These are signals of real change and appreciation for intra-continental and intercontinental partnerships.

The title of the workshop refers to An African and German perspective. Just a few years ago, this would have read A German and African perspective, with 'German' appearing first. This is another positive change. Mr Acemah thanked the German partners for noticing the changing patterns on the African continent and engaging accordingly.

Mr Acemah started his career by working on HIV/acquired immune deficiency syndrome (AIDS) and TB. He regarded the present conference

as technically stimulating and believed that participants would also leave with their spirits enriched, believing that they are part of something truly great.

Opening Remarks (Dr Anban Pillay, Deputy Director-General: National Health Insurance, Department of Health (DoH), South Africa)

Dr Pillay conveyed the apologies of the Director-General of the DoH.

The topic of public health surveillance and response to infectious diseases is opportune given the current health status in South Africa and the challenges experienced in the health system. Dr Pillay congratulated the academies on the coordination among them, which serves as a positive example and role model for the kind of collaboration that is possible between countries on issues of science in order to share research and expertise.

South Africa faces a quadruple burden of disease, as described in the *Lancet* report: the HIV/AIDS epidemic alongside a high burden of TB; high maternal and child mortality; high levels of violence and injuries; and a growing burden of non-communicable diseases. According to the statistics that South Africa reports (dating from 2015), over 460 000 deaths occurred in that year, of which 55% were due to non-communicable diseases, while communicable diseases accounted for 33%, and injuries and violence for 11.1%. In analysing these deaths, it was found that of the ten leading national causes of death, six relate to non-communicable diseases, while the other four relate to communicable diseases (HIV/AIDS, TB, influenza and pneumonia, and other viral diseases). These statistics illustrate that prevention and control of disease remain a priority for the South African DoH and the global health community.

Despite South Africa introducing free primary health care in 1994, there are still huge disparities in access to health care in the country. This is largely because of the structure of the health system in the pre-1994 period, when during apartheid there were clear differences in the way the health services were structured for different racial groups.

Improving the health system itself may not necessarily deal with many of the communicable diseases, since the contributors to these diseases include issues, such as access to safe water, sanitation, poverty, as well as the quality of education, which are key determinants in access to

good care. In trying to improve the health of all South Africans, the DoH has embarked on a number of legislative and other frameworks that are intended to improve the status of South Africans in relation to the prevention of disease and the promotion of healthy lifestyles. The aim is to provide access to health care that is equitable, efficient and of good quality.

It is important to recognise, particularly in the case of infectious diseases, that they know no borders. Politicians who would build walls between countries need to recognise that national borders do not prevent the spread of disease. Therefore, it is important to understand that multi-sectoral and multidisciplinary cross-border collaboration and coordination with active data sharing, joint health assessment, timely and transparent communication are critical. Cross-border collaboration and joint simulation exercises can be useful tools to test countries' prevention plans and enhance the collaboration and preparation for outbreaks. This cannot be addressed by one sector or one country alone. The past and recent ongoing epidemics of influenza and the Ebola virus in West Africa, the plague in Madagascar, the Zika virus in South and Central America, cholera outbreaks, malaria outbreaks, as well as the listeriosis outbreak in South Africa serve as a stark reminder of the unpredictable nature of pathogens and the importance of having resilient health systems in place. South Africa shares a vision of a world safe and secure from global health threats or infectious diseases, and recognises that in this regard it is not the capabilities of the strongest that count, but the combined effort of all. When an infectious disease overcomes the weakest among us, it overcomes all of us. South Africa is prone to epidemics of infectious diseases, such as rabies, cholera and haemorrhagic fevers.

Dr Pillay shared experiences of some critical aspects regarding South Africa's status in relation to these diseases. Cholera is one of the oldest and best-understood diseases, and strongly linked to a culture of unsafe water, poor hygiene and poor sanitation. Generally, the cases reported at health facilities represent only 10% of those infected, while 90% of cases, which are potentially infectious, remains in the community. South Africa had its first recorded cholera outbreak in 1973. In August 2000, KwaZulu-Natal experienced one of the biggest cholera epidemics nationwide in history. In July 2001, over 106 000 people were infected and 232 died. Subsequent to this epidemic, several sporadic small outbreaks were reported in several provinces, accompanied by the loss of life. In November 2008, a suspected imported case from outside South Africa resulted in a

cholera outbreak in the Limpopo province. In 2008, other provinces also reported deaths related to cholera. Some of the contributing factors include the migration of possibly infected persons from other countries into South Africa, increased travel during the festive periods, inadequate water and sanitation, unsafe water sources, and the rainy season, which exacerbate vulnerability to cholera outbreaks. Accordingly, multi-sector and multidisciplinary stakeholders are critical in ensuring mitigation against epidemics. Every epidemic affords an opportunity to learn and improve for future epidemics. The cholera epidemic taught two things: (1) epidemic preparedness and response are critical in mitigating the impact of outbreaks, and (2) a multi-sector and multidisciplinary approach is critical in order to deal with epidemics through a cross-border approach.

Emerging zoonotic diseases

Emerging zoonotic diseases have potentially serious human as well as economic impacts, and the current upward trends are likely to continue, for example, of avian influenza. Some of the lingering zoonoses are re-emerging in some regions, although they seem to attract less public awareness; these include brucellosis and canine rabies. Increasing awareness, providing information for prevention and applying strict biosecurity measures are essential. Zoonotic diseases are a result of the strong association between human and animal health. Surveillance and biosecurity are key points to ensure the control and prevention of zoonotic diseases. It is thus necessary not only to implement biosecurity rules, but it is also important to ensure that they are correctly and strictly applied. The only way to prevent public health hazards related to emerging and re-emerging zoonoses is to adapt the human and animal health systems in a harmonised and coordinated way using the One Health approach. This would expand multidisciplinary collaborations and communications with all aspects of health care for human and animal public health. Understanding the mechanisms and the underlying causes of emerging and re-emerging infections is one of the most important scientific challenges that society must face today. If these diseases are to be monitored over time, health surveillance systems will have to be put in place with high sensitivity for detection and high specificity for diagnosis. The work of ASSAf is important to support these endeavours. Cross-border mechanisms are also critical in complying with the requirements of the International Health Regulations of 2005. One of the key concerns remains the frequent travel between South Africa and other endemic areas. Extensive travel through ports of entry creates the risk of importation and exportation of diseases. For this reason, South Africa's

ports of entry, airline companies, as well as various provinces along the borders are requested to be on the alert and identify the importation of infectious diseases.

There was an outbreak of plague in Madagascar in 2017 in which 194 cases were reported (including both suspected and confirmed cases) in 20 districts across the country, with 30 deaths. An imported case of plague was reported in South Africa through the surveillance system, and it was possible to effectively identify and contain this case. During the Ebola virus outbreak in West Africa, South Africa was successfully able to prevent the importation of Ebola through the ports of entry and by implementing robust measures, such as screening for authorisation of travel between South Africa and other countries. In the spirit of humanitarian action, the South African government recruited two teams of volunteers who were deployed in the Ebola treatment centre in Sierra Leone. These volunteers were able to identify those who were ill and offer treatment. The South African National Institute for Communicable Diseases and National Health Laboratory Services developed a mobile laboratory unit, which was established in Freetown, and was a key point at which services were delivered in the Ebola-infected areas.

South Africa continues to play an important role in trying to support countries to deal with epidemics that occur outside national borders. Prevention and control of epidemics remain a priority for South Africa. It is important to appreciate the enormous social and economic consequences in a closely connected and interdependent world. It is often difficult for the Ministry of Health to get the budget that it requires to manage many of these diseases. Colleagues in National Treasury have a better appreciation of the impact of managing infectious diseases when there are economic consequences of not doing so. Another best practice is the One Health approach to manage the outbreak of various infectious diseases in South Africa.

Antimicrobial resistance strategy

South Africa has implemented an AMR strategy. Adopting and implementing the strategy are critical for government to take account of. Access to effective basic treatment to manage infectious diseases is critical to the

success of the AMR strategy. There is currently a shortage of vaccines, such as Bacillus Calmette–Guérin vaccine (BCG) and basic antibiotics, such as penicillin, largely because these are not commercially attractive for companies to produce. The consequence is that far more expensive drugs are used to manage diseases, with significant consequences for the success of the AMR strategy. It is important to focus energy on dealing with these matters.

Listeriosis

South Africa is currently experiencing the largest listeriosis outbreak recorded internationally. At a recent G20 meeting, there was considerable concern about the extent of the listeriosis outbreak. Coming just after the Joint External Evaluations in relation to the World Health Organisation (WHO) International Health Regulations, the outbreak afforded South Africa the opportunity to implement some of the recommendations. The South African DoH has published regulations on notifiable medical conditions, which now include listeriosis. The DoH has also implemented a multidisciplinary team that includes the departments of Trade and Industry; Agriculture, Forestry and Fisheries; Cooperative Governance and Traditional Affairs; the Consumer Council; WHO and others. The intention is to create an integrated approach to manage listeriosis outbreaks. The integration of services will help manage the investigation and identify the sources of this bacterial infection, recall infected products and monitor outbreaks from other sources. The outbreak called for intensive communication. The DoH appreciates the lessons it is learning in terms of developing a more resilient process.

This conference comes at a good time. Professionals have the opportunity to identify their roles and possible contribution to prevent, protect, control and provide public health responses to the international spread of disease in the light of the International Health Regulations.

The importance of cross-border surveillance, and professionals from three countries coming together at this conference, as well as the opportunity for scientists to give advice to government on infectious diseases, was emphasised.

Keynote Address I: Future Prospects in Infectious Diseases: Critical Tools to Manage Outbreaks on the Continent (Dr Izukanji Sikazwe, Centre for Infectious Disease Research in Zambia, Zambia)

The Centre for Infectious Disease Research (CIDRZ) was founded in 2001 as a collaboration between the Zambian Ministry of Health, the University of Zambia and the University of Alabama, Birmingham. Its activities were centred on clinical trials mostly for prevention of mother-to-child transmission, working mostly with nevirapine. In 2011, CIDRZ was established as an independent local organisation, with Zambians making up most of senior management and the board of directors. The centre is mostly grant-dependent (competitive or solicited), with funding from the United States (US) government (President's Emergency Plan for AIDS Relief (PEPFAR)/Centres for Disease Control and Prevention (CDC), National Institutes of Health (NIH)), European and Developing Countries Clinical Trials Partnership (EDCTP), United Kingdom (UK) Department for International Development (DFID), European Union (EU), Bill and Melinda Gates Foundation, ELMA Foundation, TB Alliance and others. CIDRZ has a footprint across four provinces in Zambia, two of which have the highest prevalence of both HIV and TB. The centre supports the delivery of high-quality health care services for hundreds of thousands of Zambians within the public health system, conducts groundbreaking research, and has become a local and international training ground and resource. CIDRZ is the biggest research institute in Zambia.

CIDRZ has a strong commitment to answering key research questions relevant to Zambia and the region. Its key activities are:

- Supporting local financial and technical ownership of high-quality, complementary and integrated health care services within the public health system.
- Facilitating clinical, research and professional development training.
- Partnering with multiple leading universities and institutions to ensure that the latest methodologies are used to answer locally relevant health questions.

Historical review of infectious disease outbreaks on the African continent

Since the 1980s, the global number of disease outbreaks has risen, while the variety of diseases has also increased. The proportion of diseases transmitted by animals and other vectors has also risen relative to those that are human-specific. The map of global emerging and re-emerging

diseases has become busier over the years. The 'deliberately emerging' infectious diseases are of particular concern. This refers to the anthrax outbreak in the United States of America (USA), where a group of individuals deliberately sent out an infectious pathogen. This is particularly worrying in the current global context. There have recently been alleged chemical releases in Syria that killed hundreds of men, women and children.

Between 1976 and 2017, the Zairian Ebola virus strain was the most prevalent. In the most recent Ebola virus outbreak in Guinea, Liberia and Sierra Leone in 2016, there were 28 616 cases and 11 310 deaths.

The drivers of infectious diseases, particularly HIV, hepatitis and TB, are clearly visible, including crowding and a lack of hygiene, but countries do not seem to be very active in controlling them. Asylum seeking is a human right, but systems have to be instituted to accommodate refugees and ensure that they have access to safe water, housing and health care. Prison systems are a source of overcrowding and infection.

Recent outbreaks in Africa

Recent outbreaks of infectious diseases in Africa include Lassa fever in Nigeria, hepatitis E in Namibia, Rift Valley fever (RVF) in The Gambia, Chikungunya in Kenya, listeriosis in South Africa and cholera in the Democratic Republic of the Congo, Mozambique, Tanzania, Somalia and Zambia.

The listeriosis outbreak in South Africa began in January 2017 and was still ongoing because of its long incubation period of 70 days. There had been 982 cases with 28% fatalities, which was one of the largest outbreaks worldwide to date. Listeriosis had been isolated from blood cultures, as well as cerebrospinal fluid. The most affected group were neonates, accounting for 41% of cases. The source of the infection was fortunately identified, although it took more than 14 months from the initial cases. Measures had been instituted to recall products and clean up the meat-processing plants responsible for the outbreak. Several neighbouring countries banned the import of meat products from South Africa. The listeriosis outbreak has been accompanied by considerable hysteria, fuelled by social media and fake news or alternative facts. Tiger Brands, the majority shareholder in the company that produced the infected products, suffered a drop in its share price.

Cholera has re-emerged in Zambia. The defence force was called in to clean up and move vendors off the streets. Some types of the seventh cholera epidemic, imported from Indonesia in the 1960s, continue to proliferate across the African continent. Cholera accounted for about 9% of cases of diarrhoea worldwide in 2013. Zambia has suffered recurrent outbreaks of cholera since the early 1990s, driven by government transition during the departure of President Kaunda and the consequent negative impact on the economy. The cholera outbreak was one of the factors that led to the wide-scale public disaffection that drove Kaunda out. The most recent outbreak began in September 2017 and is still ongoing with recent spikes in some sub-districts in the Lusaka province. The source was contaminated shallow water wells in high-density areas, mostly in the capital city of Lusaka, due to the lack of sufficient sanitation systems for the large urban population. By March 2018, there had been 4 443 cumulative cases and 82 deaths, and a case fatality rate of 1.85%. The outbreak was being treated with Ciprofloxacin, and the usual emergency management responses were being instituted to contain it.

Although the focus of the work of CIDRZ has been on HIV, the centre is involved in cholera research funded by mostly by DFID, with some WHO funding:

- Immunological characteristics of a population at risk of cholera are being profiled before and after the first and second doses of the oral cholera vaccine.
- The Extended Dose Interval Study (EDIS) determines changes in the vibriocidal geometric mean titers in subjects who receive the second dose of oral cholera vaccine at different intervals: two weeks or six months after the first dose of vaccine.
- Cholera surveillance is being strengthened through hotspot mapping.
- The relationship of cholera strains between historical and new outbreaks is being investigated.

Critical tools to manage outbreaks on the African continent

Critical tools for managing outbreaks are centred on being able to detect and monitor an outbreak and institute timely responses. A vibrant multidisciplinary workforce is required. During the listeriosis outbreak in South Africa, integrated teams were instituted, made up of different professionals including not only health care workers, but also engaging with the community. The thinking that only infectious disease physicians should be involved in managing a disease outbreak is wrong. Multidisciplinary

teams could be made up of epidemiologists, clinical microbiologists, laboratory technicians, pharmacists, public health advocates, other clinical providers and social workers. There is also a critical role for communication specialists and community leaders.

During an outbreak, the role of infectious disease physicians and clinicians is to:

- Recognise that a new outbreak is taking place.
- Collaborate with laboratory staff with respect to correct sampling and interpretation of results.
- Conduct appropriate isolation and treatment.
- Interact with public health advocates and governments to communicate correct and appropriate information.

Technology must be effectively used to extend electronic health record capabilities by providing real-time access to clinical and non-clinical data from multiple data sources, for example, coupling geographic information system (GIS) data with mobile health to map outbreak hotspots in field settings. Targeted data analytics need to be employed to detect emerging disease threats by monitoring the incidence and prevalence of conditions and pathogens. Communities must be mobilised and communication improved.

Active and passive surveillance is needed to detect infectious diseases. Infectious disease intelligence is the best defence, which entails systematic ongoing collection, collation and analysis of data for public health. Surveillance networks include:

- Global Outbreak Alert and Response Network (GOARN) launched by WHO in April 2000, which links real-time networks with data, expertise and skills needed to keep the international community alert and able to respond.
- Africa Centres for Disease Control and Prevention (Africa CDC), which started in in January 2016 and has established the Regional Integrated Surveillance and Laboratory Network (RISLNET) with five regional collaborating centres in Egypt, Nigeria, Gabon, Zambia and Kenya.
- Global Health Security Agenda (GHSA) launched in 2014 with funding from the Group of Seven (G7) countries.

Zambia recently established its first public health institute and plans to build its first public health laboratory but lacks the right laboratory support

needed in the country. Surveillance is facing funding challenges with the reduction in the work of the CDC, including its work on the African continent.

Laboratory capacity plays a critical role before, during and between outbreaks with respect to early warning signals, outbreak response and management and trend monitoring. The establishment of as many reference laboratories as possible with international accreditation in key localities across the African continent is essential. Africa has several biosafety level (BSL) 4 and BSL 3 laboratories, which play a role in early warning, supporting the outbreak response, and the monitoring and management of outbreaks. Laboratories should be well networked with country-specific focal point persons in each one to coordinate the information flow during and after outbreaks. In order to respond appropriately, public-private partnerships must be maximised. This cannot be a government responsibility alone, but close collaboration is needed between various diagnostic centres, clinical teams and public health institutions. Training initiatives and innovative programmes must be created to support exposure to infectious diseases. Resources are required to improve access to training programmes and internships, as well as improved compensation. Investment and political will are needed to support a prepared and vibrant workforce. A behaviour change approach should be utilised to limit exposure to infectious pathogens.

The challenges include:

- Surveillance systems are currently inadequate.
- There is a dwindling pipeline of new antibiotics and antivirals, continued emergence of resistant pathogens, and non-existent or ineffective antimicrobial stewardship.
- There is a lack of creative training initiatives in relation to infectious diseases with respect to emerging topics and outbreaks, undergraduate-level training, combined certification pathways (e.g. infectious diseases and clinical microbiology, or infectious diseases and critical care) and public health training.
- There is a dwindling pipeline of new infectious disease trainees entering programmes in the USA, and positions are vacant because the compensation is not equivalent to what counterparts in surgical or other medical specialisation fields receive.

- There is a lack of vaccines due the cost and long turn-around time to produce safe and effective vaccines. The Ebola vaccine is a case in point. Researchers, specialist scientists and policymakers had to move quickly to address ethical considerations, obtain financing and increase the pace of production to get the vaccine to the population.
- Resources are required to improve access to training programmes and internships.
- Investment and political will are needed to support a prepared and vibrant workforce to address the unrelenting infectious threats.
- Other cadres of health care works should support detection, monitoring and response to possible outbreaks.
- Point-of-care diagnostics must be used to detect new pathogens.
- Microbial-resistance patterns must be improved.
- Accredited clinical labs are required with functional microbiology and virology systems.
- Hubs and referral networks must be mapped.
- Human resource gaps need to be addressed.
- Communication and information systems are inadequate.

There is currently a fourth HIV epidemic driven by HIV-1 drug resistance, and primary resistance is increasing.

The future of combating infectious diseases is promising, given the environment of technology and the brilliant minds who are working on the challenges.

Keynote Address II: Metabolism and Pharmacokinetics of Anti-Parasitic Drugs: Implication for Treatment, Safety and Efficacy (Dr Collen Masimirembwa, African Institute of Biomedical Sciences and Technology, Zimbabwe)

The previous presentation emphasised the importance of response mechanisms to infectious diseases, one of which is treatment. The field of pharmacokinetics deals with the way in which the body handles medicines, as well as issues of drug safety, efficacy, affordability and access.

The African Institute of Biomedical Sciences and Technology (AiBST) focuses on drug safety, efficacy and cost/benefit analysis. The institute conducts translational research in the following areas:

- Drug metabolism and pharmacokinetics, which are major determinants of the outcomes related to safety, efficacy and affordability. When the major impediments to drug discovery and development were reviewed, it was shown that poor pharmacokinetics in the 1990s was a major reason, accounting for 40% of the failure due to the poor way in which drugs were absorbed, distributed, metabolised and excreted in the body. If these issues could be addressed, drugs could be developed with better efficacy and safety profiles. When the pharmaceutical industry introduced pharmacokinetic analysis in 2005, the failure rate due to poor pharmacokinetics was reduced to less than 10%. Drugs that were discovered and developed before the advent of pharmacokinetic integration still carry liabilities that could compromise their use. AiBST addresses these issues and what could be done to rescue the situation. The ultimate outcome of drugs depends on the complex interplay of many factors. Pharmacokinetics looks at the concentration of drugs over time, and the levels at which they are most effective against disease without side effects. In these investigations, AiBST collaborates with partners in medicinal chemistry and pharmacology.
- Pharmacogenetics, since the genetic makeup of people impacts on the way in which they respond to medicines.

The institute also conducts clinical trials.

Much of the work of AiBST is done *in vitro*, and some entails computational modelling. Animal studies are decreasing as they are not very predictive of the human response. The liver is the key organ for the breakdown of drugs, and the major determinant of the pharmacokinetics of a drug. In some cases the drug itself could be effective, but in other cases the medication, after administration, is metabolised into a pharmacologically active drug, resulting in a prodrug, and it must then be ensured that enzymes produce enough of the active compound. A danger is that the metabolites could be very reactive and result in toxicity. The major enzymes responsible for metabolising compounds are cytochrome P_{450}s, either to facilitate excretion or by bio-activation.

Most of the drugs for parasitic diseases were discovered in between the 1940s and 1970s, and there have not been new drugs since then. There are

big challenges to young people to discover new medicines, particularly for neglected tropical diseases. AiBST investigates the efficacy of drugs (amodiaquine, praziquantel and efavirenz) and ways of addressing their liabilities and reintroducing the drugs to the market.

Amodiaquine for malaria has serious neutrocytosis and liver toxicity issues, but was put back on the market after the development of widespread resistance to chloroquine. The approach was to rescue the drug through designing away from toxicophores. Isoquine had been found to be a promising analogue devoid of potential for liver toxicity, but it failed in clinical trials due to agranulocytosis. The research at AiBST began by analysing the enzymes responsible for metabolising the compounds. The metabolism of amodiaquine was then looked at in more detail. It was realised that amodiaquine was also being metabolised through an aldehyde, which was a very reactive compound circulating in the blood. Another mechanism for the toxicity was thus discovered. The initial work thus only solved the liver toxicity but not the blood toxicity. Molecular modelling was then done to try to design analogues that could bypass the bioactivation pathway. AiBST collaborated with Prof Kelly Chibale at the University of Cape Town and synthesised a number of analogues, which were tested for anti-malarial activity. They proved to be more potent than amodiaquine or chloroquine. The compounds were tested for reactivity, and it seemed that the aldehyde formation was not taking place. It seems that an analogue has been developed that has anti-malarial activity but does not carry the reactive capacity of the aldehyde. Future work would investigate whether a variant of compound 19 could replace amodiaquine in malaria combination drugs.

Praziquantel, which is used to treat a number of types of parasitic worm infections, has variable efficacy because it is rapidly removed from the body. However, it is still the only drug available to treat schistosomiasis, which affects over 207 million people in 78 countries. In countries where schistosomiasis is endemic, annual mass drug administration is done (i.e. the drug is administered to four million children within a week). There are many complaints about adverse drug reactions and poor treatment outcomes. It is the R form of praziquantel that is effective, and not the S form. The drug is administered as a mixture of the R and S forms, which means that of the 600 mg dose, only 300 mg are actually required. The research looked at what causes the R form to be metabolised and removed from the body. Rifampicin (RIF), which is used to treat TB, was found to markedly decrease

concentrations levels of praziquantel. This means that when praziquantel is administered to patients who are coinfected with TB and schistosomiasis, the praziquantel is ineffective. Concentrations of (R)-praziquantel need to be raised. The approach was to rescue the drug by using a boosted regimen. The research at AiBST was inspired by work with HIV drugs in which protease inhibitors are administered together with retinol V, the purpose of which is to inhibit the enzyme that removes protease inhibitors for sustained exposure levels. The research at AiBST began by looking at the kinetics of praziquantel and identifying compounds that could inhibit the enzymes, so that by administering them together the exposure levels of praziquantel could be boosted for better treatment outcomes. Metabolic studies have been done to identify the biotransformation routes. With that knowledge, the research team can look for other compounds that can be co-administered. It has been found that exposure levels are boosted if praziquantel is administered together with a cytochrome P450 (CYP)1A2 inhibitor. This could be a quick solution to ensure that the use of praziquantel among the population is not compromised through co-administration with a compound that ensures sustained exposure and maintenance of effective concentrations.

Efavirenz is currently the drug of choice for treating HIV but carries severe neuropsychiatric side effects, particularly in the African population. AiBST focused on how to minimise the toxicity and maximise the benefit of the drug. There is a genetic variable associated with reduced capacity to remove the drug. Higher exposure levels are associated with higher severity of the adverse drug reaction. The research found that the African population was distinct from Caucasians and Asians in terms of genetic variance for drug response, and that there are also complex variations among the African population with clinical implications. Eighty per cent of people treated with efavirenz had at least one severe adverse reaction. More than half of patients were outside the therapeutic concentration range. It was concluded that the dose of 60 mg was too high. Some of the results were published in 2008, but the government did not listen. In 2013, when efavirenz became first-line therapy and patients kept complaining, the government began to show interest in the findings. By 2016, efavirenz was ranked second among the drugs causing adverse reactions throughout Africa and a solution was required through personalised dosing. AiBST looked at their data and developed a model to try to understand how best to use efavirenz in the African population. It was shown that a patient

must be genotyped for enzyme status and those with reduced capacity to remove the drug must be given doses of only 200 mg instead of 600 mg. The weight and gender of the patient are also taken into account. The approach was to rescue the drug through use of a pharmacogenetic-based dosing algorithm.

This research gives rise to the question of whether Africa is ready for precision personalised medicine. AiBST believes that this is possible, and that Africa is ready. Efavirenz is an example that this approach can be actively deployed. Polymerase chain reaction (PCR) machines are not expensive, so the issue of cost should be removed from the equation and the focus should be on the benefits. The Zimbabwean government has asked for a cost/benefit analysis of the proposed efavirenz personalised medicine regime.

Apart from its work on rescuing drugs, AiBST is working on building infrastructure, developing technologies and training the next generation of biomedical scientists to support the whole drug discovery and development value chain.

AiBST has set up a laboratory in Harare with the capacity for genomic analysis as well as biomimetics for drug concentrations and pharmacokinetic and pharmacodynamic modelling, benchmarked against best practice in industry. Building the facilities is a slow and expensive process. A 400 m^2 phase I clinical trial unit that meets international standards has recently been set up with 28 beds, data-capture facilities, archive room, board room, offices and a research pharmacy. This facility enables AiBST to do clinical trials in partnership with pharmaceutical companies that are trying to introduce drugs into Africa.

AiBST believes that the future lies in forming strong partnerships with drug discovery and development companies so that Africa is taken into account at an early stage when the drugs are developed.

Discussion

Question: What are the statistics for resistance to HIV drugs and are there alternative drugs? What are the statistics for the prevalence of HIV type 2 in Zambia?

Response (Dr Sikazwe): There is about 12% resistance to the first-line non-nucleoside reverse transcriptase inhibitors (NNRTI) drug used in lower-income countries in the region. Local data from Zambia from 2009/10 found resistance to efavirenz to be 5 – 6%. Zambia has been working since 2010 to establish a drug-resistance surveillance system, but it has not yet been set up. The protocol is almost ready for submission, and funding is now available for primary resistance surveillance work. Zambia is getting ready to roll out an alternative first-line drug to overcome primary resistance.

Zambia mostly has HIV-1 (subtype C), and HIV-2 rates are low. There has not been any work to specifically identify the rates of HIV-2. From work done in the late 1990s and early 2000s, the rates of HIV-2 are less than 1%, and this type usually occurs in individuals who have come from West Africa.

Question (Prof Stefan Kaufmann): Is there any evidence that the activation of CYP molecules is induced by the drugs and how they are induced, mostly by praziquantel? What is the receptor of the transcription that activates the CYP molecule?

Question (Prof Axel Brakhage): Would it be possible to try to inhibit the enzyme?

Response (Dr Masimirembwa): These enzymes are mainly non-hepatic (i.e. not in the liver) but occur in the lungs or blood cells. The expression of CYP1A1 and CYP1B1 in the lungs or blood bio-activates amodiaquine, which is induced by polycyclic aromatic hydrocarbons going through the hydrocarbon receptor.

The enzymes involved in the metabolism of praziquantel are CYP3A4 and CYP2C. For CYP3A4, the pregnane X (PX) receptor is the mechanism through which induction takes place. There are many drugs that are inducers of CYP3A4; RIF is an important inducer. Nevirapine is therefore not given together with RIF because RIF will cause CYP3A4 levels to shoot up.

Boosted regimens are good clinical examples. For some P_{450}s, specific potent inhibitors are known. In the case of expensive drugs, such as cyclosporine, smaller doses could be administered together with ketoconazole, itraconazole or fluconazole, which would block CYP3A4. AiBST is looking at whether that kind of mechanism could be borrowed to boost exposure levels of praziquantel without side effects to reach levels that kill schistosomiasis.

Round-Table Discussion I: Challenges of Diagnosis and Management of Comorbidities

Moderator: Dr Izukanji Sikazwe, Centre for Infectious Disease Research in Zambia, Zambia

Panellists:
Dr Shevin Jacob, Infectious Diseases Institute, Uganda/Liverpool School of Tropical Medicine, United Kingdom
Dr Alex Sigal, Africa Health Research Institute, South Africa
Dr Jackson Marakalala, University of Cape Town, South Africa
Prof Gayle Sherman, National Health Laboratory Service, South Africa
Dr Norbert Heinrich, University Hospital, LMU, Munich, Germany

The moderator introduced the session. Africa is facing several epidemics. The epidemic of non-communicable diseases (NCD) intersects the epidemics of HIV and communicable diseases.

Dr Shevin Jacob, Infectious Diseases Institute, Uganda/Liverpool School of Tropical Medicine, United Kingdom

Dr Jacob gave a presentation on Uganda's experience with HIV and non-communicable disease comorbidities.

NCDs kill approximately 40 million people each year, accounting for about 70% of all deaths globally. The four most common NCDs are: cardiovascular (17.7 million deaths per year year), cancers (8.8 million), respiratory diseases (3.9 million) and diabetes (1.6 million). Over 80% of the deaths that are deemed 'premature' (i.e. between the ages of 30 and 69 years) occur in low and middle-income countries (LMICs).

In a quote from a 2014 article: "We are now in an era when people living with HIV may experience a reduction in quality of life or die, not from HIV itself, but from a preventable NCD that may be a consequence of HIV-related immunosuppression, antiretroviral drug toxicities, or HIV-related inflammation and hypercoagulation." Specific considerations for HIV and NCDs in LMICs include higher prevalence of infections such as TB and hepatitis.

The Infectious Diseases Institute (IDI) in Kampala, Uganda looked at 1 000 patients in the antiretroviral treatment long-term cohort between May 2014 and September 2015. This cohort comprised adults who had been on antiretroviral (ARV) treatment for ten consecutive years: 345 (34.5%) of the patients were from a pre-existing IDI research cohort and 655 (65.5%) from a routine clinic. Eighty-one per cent of the cohort was on first-line ARVs:

- AZT/3TC/NVP (44%)
- AZT/3TC/EFV (22%)
- TDF/3TC or FTC/EFV (10%).

About 21% of the cohort suffered from hypertension; fewer than 5% had cardiovascular disease (CVD) or diabetes. Almost 23% engaged in tobacco use and 73% in alcohol consumption.

The Integrated Management of Adolescent and Adult Illness (IMAI) Package of Essential Non-communicable Intervention Tools (PEN) framework has been established by the WHO to integrate NCD care within primary health centres in LMICs. Tools to implement this framework have been developed by two NGO: the IMAI Alliance based in the USA, and Walimu (co-founded by Dr Shevin Jacob) in Uganda. These tools are currently being piloted in primary health centres within Uganda through collaboration with the Ugandan Ministry of Health and include the following:

- *Acute care* involving screening/early detection for CVD risk, hypertension, diabetes mellitus (DM), and breast and cervical cancer. This focus area includes a systematic approach to acute care for severe hypertension, diabetic ketoacidosis (DKA), myocardial infarction and heart failure involving emergency assessment and then essential pre-referral treatments.
- *Integrated NCD chronic care*, with a focus on sequence of care, task-shifting and a modular approach involving three protocols:
  - Protocol 1 (HEARTS for CVD/DM): fully technically compatible with and operationalises the Global HEARTS programme. It involves training for physicians, nurses and auxiliary staff and task-shifting in the case of limited human resources to auxiliaries (nursing assistants, health educators, trained expert patients). The longitudinal patient monitoring system, which is similar to the HIV system, can support HIV-NCD co-management;
  - Protocol 2 (for asthma and chronic obstructive pulmonary disease): symptom-based management of chronic respiratory illness;
  - Protocol 3 (for rheumatic heart disease): assessment, tracking and secondary prophylaxis.

The sequence of care in integrated NCD chronic care draws on Ugandan experience of scaling up HIV care and ARV treatment. It entails clear integration of work within the clinical team and clarifies which tasks can be performed by auxiliaries, which require a clinician, and when and how to refer. It is aimed at feasible scale-up to manage a large number of patients (given the large NCD burden) and works in settings with limited human resources.

The tools developed for implementation of the WHO IMAI-PEN Protocols include:

- NCD card (similar to the HIV card used in many outpatient clinics), which provides a summary of the patient, their encounters and counselling.
- NCD register, which is a longitudinal analysis of hypertension, diabetes and elevated CVD risk.

The reports allow one to look at specific indicators and longitudinal care.

There is much research still to be done on how best to address the growing burden of NCDs in sub-Saharan Africa.

Dr Alex Sigal, Africa Health Research Institute, South Africa

At the Africa Health Research Institute, Dr Segal was involved in research on how necrosis occurs in a lung infected with TB. The findings were surprising. Macrophages perform well if they are infected with single *Mycobacterium tuberculosis* (*Mtb*) but die when they are infected by a clump. The TB is not eliminated during cell death, and will be taken up by the next cell, which will die in turn and so on. It is believed that this process of successive cells being infected and dying leads to the damage in the lung as a result of an active TB infection.

Dr Sigal's background was not as a clinician but as a computational biologist. As a basic scientist, he was gaining increasing appreciation of the importance of surveillance. It is important to be able to straddle the population. Within a population of HIV-infected individuals on antiretroviral therapy, there may be different types, for example: some may be failing therapy due to lack of adherence, while others may be partially adhering and still suppressive. The difference between these two groups with respect to the HIV reservoir is a key question for surveillance epidemiology, leading to further research.

Dr Jackson Marakalala, University of Cape Town, South Africa

Dr Marakalala is an immunologist who looks at the interaction between immunology and infectious diseases. He had done research on TB biomarkers.

In 2005/06, the first outbreak of extensively drug-resistant tuberculosis (XDR-TB) in the region occurred in Tugela Ferry. The deaths associated with this outbreak point to the lack of proper surveillance procedures being in place. There is a need for thorough preparedness to avoid outbreaks of infectious disease.

Hippocrates, who lived around 400 BC and is known as the father of medicine, observed a tumour-like structure in the lungs (now known as a granuloma). At the time, many people believed that it was hereditary and did not connect it with a causative agent. Aristotle, his fellow countryman, mentioned his belief in about 340 BC that when a consumptive coughs, something heavy in the air is transmitted to other people causing consumption (TB). However, no-one knew at that stage that there was a strong connection between a causative agent and the granuloma. A granuloma is an aggregate of immune cells that come together when the bacteria that cause TB are inhaled into the lungs. The bacteria settle in spaces in the lungs known as alveoli. Within the alveoli, the microphage cells capture the TB bacteria, and many other mononuclear cells are recruited from nearby blood vessels to the site of infection. Adaptive immunity arrives in the form of T-cells and P-cells. The bacteria are contained at the centre of the aggregate granuloma. Many people believe that the bacteria could be contained and confined at the centre of the granuloma so that people could survive without the bacteria causing disease.

Two thousand years after Hippocrates and Aristotle reported the first observations of granulomas, Robert Koch, working in in Heidelberg in 1882, cultured bacteria from a pathogen connected to TB. The Nobel Prize in Physiology or Medicine 1952 was awarded to Selman Abraham Waksman for his discovery of streptomycin, the first antibiotic effective against tuberculosis. Despite extensive research, TB remains a challenge in that 1.7 million people still die from TB each year. Sub-Saharan Africa has the highest TB burden of disease, compounded by increasing numbers of immuno-compromised individuals in the last 35 years due to HIV. Those

with HIV have a 30 times higher chance of contracting active TB due to depleted CD4 T-cells needed to structure the granuloma so as to contain TB.

One of the most important things to understand now with respect to diagnosis is how to accurately predict the disease activation from latent to active status. Granulomas that solidly contain bacteria are used to see how the structure dissolves to form caseations (i.e. cheese-like structures with cavities in the lung) through which the bacteria can be spread throughout the lung and coughed out. Research looks at the spectrum of disease at tissue level that mirrors disease progression and tries to identify molecules at each step that could help predict disease progression and be used in point-of-care diagnostics. However, not everything present in the lung circulates in the blood.

An exciting study at the South African Tuberculosis Vaccine Initiative (SATVI) at the University of Cape Town is conducting longitudinal studies for 800 days before diagnosis. Molecules have been identified that reliably show latent infection. Some of these molecules are present in blood signatures. This avenue can be explored for developing diagnoses that would catch people on the brink of developing active TB for better interventions.

Prof Gayle Sherman, University of the Witwatersrand, Centre for HIV & STI, National Institute for Communicable Diseases, South Africa

The presentation would highlight diagnostic challenges of rolling out the early infant diagnosis programme for HIV (i.e. in the first 18 months of life). It is fairly easy to diagnose adults with HIV; by taking a drop of blood from the finger and using a rapid test, an individual can be diagnosed within one to 20 minutes. Infants at risk are born to mothers who are already HIV-infected; through passive immunisation, those babies will test positive at birth regardless of whether they are infected or not if an antibody test is used. For diagnosis of HIV during the first 18 months of life, a PCR HIV viral detection assay is required, which is much more costly. Approximately 300 000 of the one million babies born in South Africa each year are born to HIV-infected women. It is essential to identify infected infants for treatment and to monitor the prevention-of-mother-to-child-transmission (PMTCT) programme.

The first challenge faced in around 2000 was 'it can't be done' due to the technology being costly and requiring considerable expertise in a

low-resource setting. The view was to rather use clinical skills and/or other biological markers with suboptimal positive predictive values to make diagnoses. When South Africa implemented the roll out of ARVs in 2004, the Antiretroviral Roll Out Guidelines did not include HIV PCR as a way of diagnosing infants.

The next challenge is to demonstrate how it can be done. This entailed developing protocols, raising funding and showing that HIV PCR testing in a local setting, albeit used less frequently than in the developed world, can achieve a definitive diagnosis, improve care and is affordable. In 2006, HIV PCR testing was adopted for other low-resource settings by the WHO in HIV Early Infant Diagnostic (EID) guidelines.

The next challenge was that of implementation. It had been overlooked that it is difficult to get a blood sample from a six-week-old infant, and there are not enough people trained to do so. In order to address this, new studies had to be done on how to do the diagnostics on dried blood spots from a heel prick, for which there is experience. After the introduction of dried blood spot testing in 2005, EID could be scaled up in locations other than tertiary hospitals where it was traditionally being done. Additional issues arose in how to deal with dried blood spot tests in the laboratory. The next challenge was to train people to conduct the diagnoses.

The early infant diagnosis programme for HIV is monitored using laboratory test data from the National Health Laboratory Service, where all data go into a single data warehouse. From 2004 when only about 2 000 HIV PCR tests were done, usually as part of research studies, the number of children under the age of two months who were tested with PCR rose to 450 000 by 2015. As PMTCT was rolled out in South Africa, there was a decrease in mother-to-child transmission to only 1.8% in 2014. This was a year ahead of the target of below 2% set in the National Strategic Plan (NSP) for 2015.

Other challenges remained. The HIV epidemic had been evolving; there was more ARV drug pressure; more sensitive HIV PCR assays became available; and there was more knowledge about the poor outcomes that infants infected with HIV experienced. These factors led to the introduction of new HIV EID Guidelines in 2015.

New truths about HIV emerged. It used to be taught that once an HIV PCR was positive in a child, it would remain positive; however, this is no

longer the case. With the ARV drug pressure, can be more difficult to make a definitive diagnosis of HIV in children now than it was 15 years ago because of lower viral reservoirs and/or viral loads. Clinicians have to deal with many indeterminate tests in which they are not sure whether the patient is HIV-positive or not.

Dr Norbert Heinrich, Division for Infectious Diseases and Tropical Medicine, University of Munich, Germany

Dr Heinrich gave a presentation on TB diagnostics and sequelae.

According to WHO statistics, there are ten million new cases of TB per year, but of these an estimated four million cases are not reported to the health system, do not receive diagnosis and treatment opportunities, and continue to spread the disease. Without treatment, mortality is about 50% within two years. In the past, microscopy was the only diagnostic tool with widespread availability; and its high limit of detection of 10^5 TB bacilli per millilitre of sputum, often contributes to missed or delayed diagnosis. Other factors also played a role in late diagnosis, including access to health care. Delay in diagnosis makes the greatest contribution to morbidity, mortality and transmission.

A lung function impairment study in a Maputo cohort found that about one-third of patients still had some lung impairment in spirometry at the end of their treatment. The long-term consequences in terms of sequelae are not well researched.

The diagnostic situation for children with TB is difficult. Respiratory samples are difficult to obtain, especially in small children, due to low volumes and low bacterial burden, which are sub-optimally sensitive. In terms of TB epidemiology, there are one million new cases with 209 000 deaths annually

A number of new approaches are being investigated, ranging from PCR to biomarker signatures and immunological tests. WHO has developed target product profiles for new tests:

- A point-of-care biomarker test.
- A point-of-care triage test.
- A point-of-care sputum-based test to replace smear microscopy.
- A rapid drug-susceptibility test at the microscopy-centre level.

The rapid and accurate diagnosis of paediatric (RaPaed) TB project is about to establish a paediatric diagnostic consortium to validate new child-friendly tests among a cohort of 800 children with TB symptoms.

Discussion

Question (Dr Collen Masimirembwa): Are dry blood samples being bio-banked for other research purposes?

Response (Prof Sherman): There were initially efforts to biobank samples, but samples are now discarded according to a routine programme within a few weeks to a year, depending on how much space the laboratory has available. The samples from a South African PMTCT evaluation study over three years, which produced 10 000 dried blood spot samples, is looking for a home.

Response (Dr Segal): The Africa Health Research Institute has a demographic surveillance site where the individuals who are being monitored are asked for blood spots at least once a year. This is a valuable resource not only for DNA sequences of the donor but also viral sequences and levels of antiretroviral drugs including tenofovir phosphate, for example, which indicates adherence. There needs to be consideration of bio-banking samples, because there is potential to return to the individuals and see how successful the outcomes were.

Question: What is the correlation between babies that are born HIV-positive serologically and those who are found to be PCR positive?

Response (Prof Sherman): The mother-to-child transmission rate is currently less than 2%, which means that almost all the babies would be serologically positive at birth and they would gradually lose maternal antibodies. Up to the age of about six weeks, fewer than 2% would be PCR positive for HIV infection.

Question (Dr Sikazwe) enquired about the implementation of integrated clinics and how many patients they were seeing.

Question: How do you intend to monitor an HIV-infected population?

Response (Dr Jacobs): The work on integration is funded by the WHO Regional Office for Africa (WHO-AFRO). It is currently being piloted in five health care centres, which are relatively small units that see about 1 400 patients with NCD over a three-month period. With respect to feasibility, the main question in the context of diminished human resources for health is how to manage patients with a burden of HIV disease inside and outside the HIV context. Of the five health care units, only one is specifically an HIV clinic, but all are using the same model that has been used in Uganda for HIV care.

Question: Are granulomas an indicator for preventative action?

Response (Dr Jackson): When patients are treatment refractory (i.e. they do not respond to currently available drugs) and there is severe damage to the lung, they generally undergo surgery to remove infected tissue from the lung. When the granuloma is still intact, latently infected individuals tend to have a disease that is well contained. Only 5 – 10% of infected people with bacteria in the lungs develop active TB. The presence of bacteria in the lung provides the potential for granuloma to develop, within which the disease may be protectively contained. The disease is only spread through the lung if the granuloma structure dissolves to form caseations.

Question (Dr Sikazwe) enquired about experience of disease progression in other comorbid conditions among HIV patients.

Response (Dr Jackson): With respect to comorbidity, emerging data show that lifestyle diseases such as diabetes triple the chances of developing active TB. The high inflammation that may occur when a patient is taking medication for both HIV and TB also increases the chances. For effective projection of the outcome of treatment, it is necessary to understand the patient and the underlying diseases.

Question (Dr Sikazwe) asked whether microphages are killed in the same way in patients with drug-sensitive or resistant TB.

Response (Dr Segal): This has not yet been tested. XDR and MDR-TB strains are difficult to make fluorescent. From a clinical perspective, XDR and MDR infections do not seem to be completely cured with chemotherapy, but the individuals can still function for a while.

Response (Dr Heinrich): There is a question of whether MDR is generated through failing treatment or transmitted. Modelling studies have shown that two-thirds of MDR is transmitted, not generated. In these circumstances, it is unrealistic to hope that MDR will subside just by optimising first-line treatment in those demonstrating resistance.

SESSION 2: ANTIMICROBIAL RESISTANCE

Facilitator: Prof Charles Wiysonge, South African Medical Research Council, South Africa

Keynote Address III: Antibiotic Resistance in Africa: Challenges and Strategies (Prof Sabiha Essack, University of KwaZulu-Natal, South Africa)

There are controversies around antibiotic resistance related to the over-use of antibiotics in livestock and fish farming, poor infection prevention and control in hospitals and clinics, lack of hygiene and poor sanitation, poor coverage of vaccination within communities, as well as the lack of new antibiotics in the pipeline. Of the new antibiotics undergoing clinical trial, none have a novel mechanism of action. The current approach is mainly to look at combination therapy and repurposing older drugs.

Antibiotic resistance happens when bacteria change and become resistant to the antibiotics used to treat the infection they cause. It occurs as the result of selection pressure with the warranted and justified use of antibiotics, as well as indiscriminate use or misuse. The greater the exposure to antibiotics, the greater the chance of mutation to become resistant to the antibiotics. Antibiotic resistance can be spread via the food chain or from person to person. The time period between the introduction of a new antibiotic and the development of resistance is reducing. In the case of antibiotics introduced since 2000, antibiotic resistance developed within less than five years in the case of Linezolid and Daptomycin. Very few antibiotics have enjoyed a long period of efficacy in the clinical setting.

Antibiotic resistance (ABR) has extensive health, economic and societal implications. For the last three years, the *Global Risk Report* published annually by the World Economic Forum has identified ABR as a threat to the world economy. The implications of ABR are that infected people are sick longer, stay away from work longer and have a greater potential of spreading the infection so that more people get sick. Not only does the individual suffer loss of income, but also the industry. ABR knows no borders and affects both developed and developing countries.

A review of AMR by economist Jim O'Neill commissioned by the UK prime minister found that unless AMR is addressed by 2050, there will be ten

million deaths as a result of AMR with an international cost of US$10 trillion. The implications are that even a simple ear infection will not be able to be cured with antibiotics. A disproportionate mortality rate is anticipated for Africa and Asia at the epicentre of AMR: Africa (4.1 million) and South East Asia (4.7 million).

Because of the implications of ABR, it has generated unprecedented political attention among the G8, G20 and G77, and it even featured in the UN General Assembly in 2016. This was only the fourth time that a health issue was tabled at the UN. The first occurrence related to HIV, the second to NCD, the third to Ebola, and the fifth occurrence will be TB in 2018. The UN formed a task force comprising an alliance of several important global role players to address AMR. The UN has endorsed the Global Action Plan on Antimicrobial Resistance, a tripartite alliance between the WHO, Food and Agriculture Organisation (FAO) and the World Organisation for Animal Health (OiE), which have committed themselves to addressed AMR collectively because antibiotics and other AMRs are used in all three sectors of human, animal and environmental health. The environmental component is somewhat lacking but does have an important role to play especially with respect to the disposal of AMRs or the use of animal litter to fertilise agricultural soil.

AMR or ABR has been described as the quintessential One Health issue because of its prevalence and transmission between the three sectors of human, animal and environmental health. As long ago as 1977, there was awareness that AMR was a complex issue that could only be effectively addressed through a holistic approach as opposed to focusing on each sector individually. The huge investment by pharmaceutical companies in the development of new antibiotics has waned and few pharmaceutical companies are currently working in this area.

An alternative to the epidemiological framework is the Collective Action and the Risk Ecosystem (CARE), which takes the perspective of exposure. People are exposed to AMR through various interactions with food, water, wild animals, companion animals, sewage, rivers, pesticides, hormone and other compounds, pests, impact of travel, climate and weather.

Africa is the leader with respect to causes of death due to HIV, TB, malaria, diarrhoeal diseases, lower respiratory infections and other infections. AMR

confounds the successful management of infectious diseases. The collateral damage of HIV occurring together with other communicable diseases has been mentioned, but there is collateral damage related to HIV and AMR. An HIV patient has a compromised immune system and is thus more prone to infections. The higher the rate of infection, the more antibiotics are used, which increases selection pressure and the further development of ABR. The collateral damage of HIV is thus ABR. Capacities related to surveillance of antimicrobial use and resistance, infection control, drug legislation and distribution, veterinary sciences and health economy are often limited in sub-Saharan Africa. It is not possible to motivate huge investment in a surveillance system when there is little understanding of AMR. Death registers may state that a patient died of TB, pneumonia or a bloodstream infection, but it is seldom known whether the infection was caused by resistant bacteria.

WHO has been working on AMR since 1959, but despite the AMR Strategy of 2001, there has not been much uptake by countries to take substantial steps to address AMR. Only in 2011, with the adoption of the WHO stance of "no action today no cure tomorrow" did political will start to quicken. WHO published a six-point policy package, which required governments to start taking AMR seriously. In 2014, WHO published the *Antimicrobial Resistance Global Report on Surveillance* in various regions. All these initiatives have been focused on ABR. This does not mean that other microbial resistance is not important, but ABR programmes have enjoyed considerable investment. The Global Action Plan and other initiatives focused on common causative organisms of community and hospital-acquired infections.

Since 2016, implementation guidelines have been published as well as the Global Framework for Development and Stewardship to Combat Antimicrobial Resistance, the WHO Methodology for a Global Programme on Surveillance of Antimicrobial Consumption, and a revised WHO Model List of Essential Medicines. The list provides new advice on which antibiotics to use for common infections and which to preserve for the most serious syndromes, based on a thorough review of all essential antibiotics. Intended to optimise antibiotic use and reduce antibiotic resistance without restricting access, the list categorises antibiotics into three groups: Access (for treating common infections), Watch (for potential development of resistance) and Reserve (for treating serious syndromes).

The WHO Global Surveillance Report published the following data on AMR in Africa:
0–87% to third-generation cephalosporins in *Escherichia coli* (ESBL+);
0–98% to fluoroquinolones in *E. coli*;
8–77% to third-generation cephalosporins in *Klebsiella pneumoniae* (ESBL+);
0–4% to carbapenems in *K. pneumoniae* (CRE);
0–100% to methicillin in *Staphylococcus aureus*;
1–100% to penicillin in *S. pneumoniae*;
0–35% to fluoroquinolones in non-typhoidal *Salmonella*;
0–9% to fluoroquinolones in *Shigella* spp.;
0–12% to third-generation cephalosporins in *Neisseria gonorrhoeae*.

The country situational analysis for Africa showed that:
- eight out of 47 member states in the region participated;
- all eight countries stated that resistance to treatments for malaria and TB are their greatest challenges;
- poor-quality medicines are a general problem, further contributing to the spread of AMR;
- data are incomplete due to lack of information;
- the results suggested that AMR is a growing problem.

A paper co-published by Prof Essack in the *Journal of Public Health* in 2016 looked at how member states in the African region are performing on the WHO Policy Package, but many African countries did not submit data on all the aspects:
- **Availability of national action plans (NAPs) to combat AMR, with aims, objectives, funding sources, responsibility, accountability and timeframes:** The report found that 38% (18/47) countries have developed NAPs and 40% (19/47) have NAP development in progress.
- **Percentage of member states in which reports on surveillance for AMR had been prepared in the last five years:** 23% (11/47) have enrolled in the Global Antimicrobial Resistance Surveillance System (GLASS); 17% (8/47) countries have initiated GLASS enrolment; no African countries have representative national surveillance systems. South Africa has a national laboratory-based surveillance programme on selected bacterial and fungal pathogens. Several countries have instituted pilot surveillance projects.
- **Access to quality medicines:** 85.1% (40) countries have access to quality essential medicines and expert opinion-based treatment guidelines.

- **Infection prevention and control policies (IPC):** 14.9% (7) have national IPC policies, which are crucial to prevent the dissemination of any infection, especially in relation to AMR infections.
- **Rational medicine use:** 87.2% (41) have rational drug use policies.

Two non-profit organisations have been working on AMR in Africa:

- Centre for Disease Dynamics, Economics and Policy (CDDEP), which is part of the Global Antibiotic Resistance Partnership (GARP). It is US-based and funded by the Bill and Melinda Gates Foundation. GARP advances policy analysis and policy development capacity in AMR. GARP chapters are national multi-sectoral working groups in low and middle-income countries. The aim is to conduct situational analyses on antibiotic use and resistance in humans and animals to inform evidence-based, country and context-specific interventions to preserve the effectiveness of antibiotics, decelerate the spread of resistance, and ensure access to antibiotics. GARP is active in Kenya, Mozambique, Namibia, Tanzania, Uganda, South Africa and Zimbabwe.
- ReAct Africa is Swedish-based and brings together experts and key stakeholders to form technical working groups on AMR. It provides technical assistance in the development and implementation of NAPs, and raises awareness of AMR among the general public and the health, veterinarian and agricultural sectors. ReAct has a toolbox that speaks to various ways in which ABR can be addressed, including awareness, surveillance, education and policy. ReAct facilitated Ghana's National Policy on AMR; partnered with the Global Alliance against Chronic Respiratory Diseases (GARD) and CDDEP GARP to support the NAP process in Zimbabwe, Zambia and Rwanda; and also partnered with GARP-Kenya.

CDDEP GARP and ReAct are now discussing how they can work together rather than duplicating their efforts by working in the same countries.

The UK government has committed £265 million to the Fleming Fund until 2020/21 in order to build capacity in surveillance networks and AMR response capacity in low and middle-income countries. AMR plays a role in Universal Health Coverage, International Health Regulations, in achievement of the Sustainable Development Goals and in the Global Health Security Agenda. These are timebound external funds, and it is incumbent on African governments to take responsibility for AMR surveillance and investing in changing behaviour and antibiotic use.

Antibiotic resistance is a tragedy of the commons, in that individuals act independently and in self-interest to the detriment of the best interests of a whole group by depleting a common resource. Antibiotic conservation requires coordinated, multi-pronged, multi-stakeholder, multidisciplinary partnerships underpinned by national and international policies that suspend sectoral interests for the public good.

Long-Term Bedaquiline-Related Treatment Outcomes in Patients with Extensively Drug-Resistant Tuberculosis from South Africa (Dr Olatunde Olayanju, University of Cape Town, South Africa)

The global burden TB amounted to 10.4 million cases reported in 2016. It is the leading infectious cause of death worldwide. In 2016, there were 1.7 million estimated deaths from TB. Drug-resistant TB continues to threaten TB control, with 600 000 new cases of rifampicin-resistant (RR-TB), of which 490 000 were multiple drug resistant (MDR)-TB reported in 2016. Approximately 10% of global MDR-TB strains are XDR-TB.

In South Africa in 2016, 3.4% of all notified TB cases were MDR/RR-TB, and 8.1% was XDR-TB. It was estimated that M/XDR-TB will consume over 80% of TB treatment cost in South Africa in 2017/18 despite MDR-TB making up less than 10% of the total caseload.

The treatment outcome is still poor due to lack of effective treatment. The culture conversion rate in patients with XDR-TB between 2002 and 2008 in South Africa was only about 19%. Seventy per cent of XDR-TB patients dies within five years of diagnosis. In 2017, WHO estimated the favourable outcome for XDR-TB in South Africa to be 27%. Patients who failed treatment are discharged home. Migration to developed countries continues unabated. The EU urged members to take in 160 000 refugees between 2015 and 2017.

Primary transmission of XDR-TB has been proven. New and repurposed bactericidal drugs such as linezolid (Lzd), delamanid (Dlm) and bedaquiline (Bdq) have offered new hope for patients with XDR-TB. A phase II trial on Bdq reported a cure rate of 61%, 6.8% mortality, and significant adverse events including QT prolongation and hepatitis, raising concerns about efficacy and safety. Long-term prospective outcome data comparing XDR-TB regimens, with and without Bdq, from an endemic setting are lacking.

A study was undertaken to compare long-term treatment outcomes between XDR-TB patients who received a Bdq-containing regimen and those who did not. The study followed up 272 patients with laboratory-confirmed XDR-TB; 204 patients received a non-Bdq-based regimen, while 68 received a Bdq-based anti-TB regimen. All patients were admitted to Brooklyn Chest Hospital, Cape Town. Demographic and clinical information were obtained by a trained health care worker. The treatment outcomes that were evaluated were cure/treatment completion, deceased, treatment failure, treatment default and lost to follow-up.

Multivariate analysis of patients in both groups suggested that receiving Bdq ($p=0.05$; HR=0.24) was an independent predictor of survival. Patients who were HIV-infected ($p=0.02$; HR=1.51) and those who weighed less than 50 kg at admission ($p<0.001$; HR=1.96) were more likely to die.

The Bdq-based regimen resulted in substantial and remarkable improvement in outcomes in patients with XDR-TB. The Bdq survival effect remained significant in HIV-infected persons irrespective of CD4 count. Initial fears about Bdq adverse events were not substantiated. These data make a strong case for the accelerated roll-out of Bdq-based regimen for the treatment of XDR-TB in endemic settings.

Discovery of New Anti-Infectives from Fungal and Bacterial Sources (Dr Kathrin Wittstein, Helmholtz Centre for Infection Research, Germany)

AMR is an increasing problem worldwide, while the number of new drugs has been decreasing over the last few decades. The pace of new discoveries in the field of anti-infectants has slowed down, especially since many large pharmaceutical companies have stepped back from this research because of the low potential for profit. Many of the drugs launched in this field over the last 30 years are mainly analogues, derivatives of already-established compounds with improved pharmacokinetics or a broader spectrum of activity. However, the evolution of resistance against these analogues is much faster because of cross-resistance. Compounds with new mechanisms and modes of action are needed. This is not an easy task. Almost 80% of anti-infective agents are still derived from natural sources, mainly bacteria and fungi. The compounds have to be chemically optimised to fulfil the requirements of a drug, but it seems that nature is still a very effective source for providing diverse new lead structures.

The presentation would focus on efforts of the Department of Microbial Drugs at the Helmholtz Centre for Infection Research to identify potential new lead structures from fungal and bacterial strains.

The screening for new bioactive compounds is a very work-intensive and time-consuming process. Redundancies are a big issue. To avoid the isolation of already-known compounds, the focus is on new species from different kinds of natural habitats that have not been well investigated. For example, the research team recently started to work on spider-associated fungi. Another option is to look at genomic data if available to check biosynthetic gene clusters in order to identify a particular strain for selection. The pre-selection of strains is crucial. Selected strains are cultivated in various liquid media as well as solid media in varying conditions. This is a key step to the production of diverse secondary metabolites in microorganisms. Cultures are extracted by organic solvents, and the crude extracts are investigated in different biological assays, mainly against Gram-positive and Gram-negative bacterial and fungal strains. In parallel, the strains are being investigating using high performance liquid chromatography – ultraviolet/ mass spectroscopy (HPLC-UV/MS) dereplication. This provides specific characteristic data of each compound in the extract mixture, which can be compared with internal and external databases to identify already-known compounds and to evaluate whether it is worth working on the extract. This is a bio-activity guided approach, but the focus is also on chemical diversity.

New antifungal compounds from Favolaschia

The *Favolaschia calocera* species is a good example, obtained from cooperation with Kenya. The fungus provided extracts with strong and selective anti-fungal activity. The mixture was fractionated into a 96-well plate using HPLC, and afterwards the test organism was applied, in this case *Candida tenuis*. In this way, there is direct correlation of activity and a specific peak in the HPLC chromatogram. For the isolation, the focus was only on the active compounds. In order to have enough material for the isolation of single compounds, the replication process has to be scaled up. This is followed by purification using chromatographic methods, mainly preparative HPLC, and structure elucidation and characterisation of pure compounds using NMR microscopy and further analysis of the purified compounds. It was possible to isolate 18 new compounds from the species, among which were four new strobilurin derivatives, which are responsible for antifungal activities. Strobilurins are a known class of

antifungal compounds. It was also possible to isolate four fairly uncommon chlorinated metabolites, which showed cytotoxic effects on mouse cells and human cancer cells.

New fungal species with nematicidal metabolites

There is also a search underway for new fungal species with nematicidal metabolites. Recently, active compounds were obtained from a new species collected in northern Thailand. The fungi will be screened on culture plates together with nematodes, and the corresponding extracts will be tested in solution on 24-well plates. The collecting trip to Thailand was very productive and several new species were discovered (one of which represents a new genus). Regular exchange takes place between the Helmholtz Centre and two institutes in Thailand (BIOTEC and Mae Fah Luang University). The new fungal genus and species, *Pseudobambusicola thailandica*, was isolated from a plant twig. Extracts show nematicidal and antifungal activity. From this fungus, six new metabolites and two known ones with nematicidal activity were isolated.

Natural products with anti-biofilm activity

Through internal collaboration, several groups at the Helmholtz Centre are investigating natural products with anti-biofilm activity in order to find new natural products with anti-biofilm activity. Biofilms are large colonies of bacteria (e.g. staphylococcus) that protect the bacteria and make them less susceptible to drugs and combative organisms. A compound that inhibits the formation of biofilms, while at the same time not interfering with bacterial growth, is unlikely to induce resistance, or at least very slowly, because it does not directly alter the phenotypes. Such a compound is a valid potential alternative to classical antibiotics. The researchers are particularly interested in quorum sensing, the communication of single bacteria during the formation of biofilm.

More complex bioassays cannot be used in the initial bioscreening. Submissions to the internal natural product library recently obtained two hits. One of these was microporenic acids A and B, isolated from the Kenyan fungi, which inhibited the biofilms of pathogenic *Staphylococcus. aureus* and *C. albicans*. The other was erinacine C (ErC), which was evolving a protection mechanism against *S. mutans* biofilms, which are present in dental plaque, for example. Mutacins were being produced, which are small antibiotic peptides that kill competitors of *S. mutans*. ErC inhibits the

production of three mutacins (IV,V,VI), weakens the biofilms of *S. mutans* and makes them susceptible to competitor organisms.

Large-scale production

If a new metabolite with particularly strong activity is discovered, the next step would be up-scaling of fermentation. Such projects usually involve strain optimisation for a specific metabolite and media optimisation. The cultivation parameters have to be transferred from screening shaking flasks to large bioreactors, which is a difficult process. Large-scale fermentation produces kilogrammes of biomaterial that has to be processed. The purification process also has to be optimised.

Corallopyronin A: novel antifilarial drug candidate

Currently, the Department of Microbial Drugs at the Helmholtz Centre is producing one compound on a very large scale, namely Corallopyronin A, which was isolated in 1985 from the soil myxobacterium *Corallococcus coralloides* and is effective against some Gram-positive bacteria. It has the same target as RIF but not the same mode of action. RIF binds to the active site of DNA-dependent ribonucleic acid (RNA) polymerase, whereas Corallopyronin A binds to the outside region that is responsible for opening and closing the active site of the enzyme. Corallopyronin A is therefore still active against RIF-resistant *S. aureus* strains. Corallopyronin A is being investigated as a potential agent against diseases caused by filarial nematodes such as river blindness. Two drugs are currently used for treatment: ivermectin which kills the nematode, and doxycycline which kills the *Wolbachia* bacteria associated with these nematodes. If the *Wolbachia* bacteria are killed, the nematodes are sterile and not able to reproduce, and they will gradually die. Doxycycline is effective, but problematic for pregnant women and children. Corallopyronin A also decreases the population of *Wolbachia* bacteria. Collaboration partners were able to show that Corallopyronin A demonstrates very significant *in vivo* activity, even higher than doxycycline, depleting and blocking the development of *Wolbachia*. The Department of Microbial Drugs at the Helmholtz Centre has established a large-scale fermentation process and thus far isolated 20 g of the natural products.

Cystobactamids: novel broad spectrum antibiotics

Another collaborative project in which the Department of Microbial Drugs at the Helmholtz Centre was involved was on cystobactamids, a new class of antibiotics with strong activity against Gram-positive bacteria as

well as some Gram-negative strains in the micro and even nano range. Cystobactamids was discovered through LC-HRMS-assisted bioactvity-guided screening. The target was identified as DNA gyrase. The binding pocket has only partial overlap with the quinolone antibiotics. There is thus a good chance that resistance would develop very slowly. The challenge in this project was the low concentration. After the identification of the biosynthetic gene cluster, alternative myxobacterial strains were used, which were also producing derivatives of cystobactamids. By combining genomic data, it was possible to identify a strain that was producing derivatives in slightly larger amounts. A new derivative was isolated which is also active against *P. aeruginosa*. This kind of activity has not been observed before. Cystobactamids are being investigated *in vivo* to evaluate their potential for further development.

Towards the Development of the Next Generation of Antibiotics from Fungal Sources (Dr Patrick Arthur, University of Ghana, Ghana)

The research activities are in the area of chemical systems biology of infectious pathogens including infection biology of tuberculosis, chemical biology of fungal metabolites, advanced imaging techniques and mass spectrometry-based proteomics of the perturbations caused in infectious pathogens by novel bioactive compounds. The research is aimed at the discovery and development of new antibiotics against tropical infectious diseases. Towards the discovery of novel anti-infective agents, the research group has isolated and screened more than 3 000 distinct fungal species. The surprisingly high rate of the occurrence of anti-infective activities in the culture extracts of these fungi has led the group to develop a phenotypic array-based workflow as a key strategy. This allows for the comparison of the bioactive extracts with many standard antibiotics on the basis of their pattern of activities across different physiological conditions of the test organisms. The group is currently in the process of isolating pure compounds from fungal extracts from the priority list. These compounds will be extensively characterised using NMR-structural elucidation, advanced imaging techniques and mass spectrometry-based proteomics to provide understanding of their mechanism of action to support preclinical development.

The research was funded by German Academic Exchange Service (DAAD)-Germany, Grand Challenges Canada, The World Academy of Sciences (TWAS)-Italy, the International Foundation for Science (IFS)-Sweden and

the Gates Foundation/Noguchi Memorial Institute for Medical Research (NMIMR).

Discussion

Question (Dr Collen Masimirembwa): Once the molecules have been identified, what strategy does the research group at the Helmholtz Centre use to decide whether to take the approach of synthesis or fermentation? Is the target differentiation from docking experiments or co-crystallisation data?

Response (Dr Kathrin Wittstein): The target differentiation was done by cooperation partners, and she could not give details of how they proceeded. Regarding the strategy, her research group is interested in bioactive compounds, especially new compounds with new modes of activity. They usually work on small molecules. The synthesis is done by cooperation partners.

Question (Dr Collen Masimirembwa): Screening provides numerous hits, but these are not being translated into new drugs. Is there a target product portfolio that guides Dr Arthur's research and provide a sense of progression?

Response (Dr Patrick Arthur): There is no organisation that guides the process of drug development. In 2015, Dr Arthur attended at TB drug discovery conference in Italy, where the keynote speaker had led the development of a drug by Johnson & Johnson that took a frustrating eight years, during which he had almost abandoned the work. The scientific world has not worked out how to guide the process of drug development in a logical manner.

Question (Dr Collen Masimirembwa): What provides confidence to pursue a 'black box' approach using a whole organism, without a molecular mechanism to guide optimisation?

Response (Dr Patrick Arthur): Their usual approach is to sample about 20 compounds in one assay. Any extract that passes all 20 is considered a top hit. The work focuses on those that maintain or improve the activity under tried and proved conditions. In this way, confidence is gained to proceed with product isolation.

Question: Dr Olayanju's research found that bedaquiline does not have side effects, but according to the literature it has considerable toxicity. How big was the sample to reach the conclusion that reported toxicity to be false? How are patients who weigh less than 50 kg treated?

Question: Given the positive effects of Dr Olayanju's research, have there been any efforts to include the new drugs in treatment?

Question (Dr Norbert Heinrich) asked Dr Olayanju's views on potential biases in his research (e.g. the non-randomised way in which the data are generated) that might lead to the conclusion that bedaquiline is effective. He would like to believe the data but is sceptical.

Comment (Prof Charles Wiysonge): Before any recommendations can be made on therapy and treatment, there has to be a randomised trial and systematic review.

Question (Dr Olatunde Olayanju): All patients in Cape Town diagnosed with XDR-TB are referred to Brooklyn Chest Hospital for treatment. When they show improvement as evidenced by culture conversion, chest X-ray improvement and general wellbeing, they are discharged to day-hospitals closer to their place of residence where the treatment continues. All patients referred to Brooklyn Chest Hospital qualified for recruitment into the study. Participants were recruited between 2008 and 2016. Because of the sporadic nature of outpatient care after discharge, it was difficult to get a large group to consent to participate in the study. Only patients who have completed 24 months of treatment were included in the study, several other patients were exempted due to this reason.

With respect to toxicity, in randomised clinical trials as well as short-term studies mostly for six months, the major adverse effects reported were mild toxicity or mild QT interval. In the study of patients who completed 24 months of treatment, no major toxicity effects were observed. Only seven had a marginally prolonged QT interval of 450 – 470 ms, but not so high as to recommend discontinuation of the treatment. None of the patients had a QT interval as high as 500 ms.

With respect to weight, multivariate analysis, showed that patients who weighed less than 50 kg were more likely to die within the whole cohort of 272. This finding was not limited to patients who received the Bdq-based anti-TB regimen.

Facilitator: Prof Sabiha Essack, University of KwaZulu-Natal, South Africa

Antibiotic Resistance and Molecular Epidemiology of *Shingella* Isolates from Children under Five Years in Manhiça, Southern Mozambique (Mr Delfino Vubil, Manhiça Health Research Centre, Mozambique)

The Manhiça Health Research Centre (Manhiça HDSS) is a biomedical research centre in a rural area in southern Mozambique that conducts research in several areas including malaria, HIV, TB, respiratory infections, diarrhoeal diseases and others, clinical and molecular epidemiology and pathology, assessment of drugs and vaccines, monitoring and evaluation, and social science projects among the community. The population of the study area of Manhiça HDSS comprises 184 000 people living in 43 000 households.

Among the thematic areas researched at Manhiça HDSS, diarrhoeal diseases continue to be the most common in children, and are the major causes of infant mortality in developing countries. Species of *Shigella* remain among the main causes of childhood diarrhoea in both developing and developed countries. Annually, 165 million cases of shigellosis were estimated to occur worldwide, with over one million associated deaths. The burden of disease due to *Shigella* is partially explained by the diversity of *Shigella* species, with more than 50 serotypes. *S. dysenteriae, S. flexneri* and *S. sonnei* are endemic. There is no effective vaccine against *Shigella*.

Antibiotic resistance is increasingly reported worldwide in relation to the most commonly used antibiotics, including ampicillin, tetracycline, chloramphenicol, sulfonamides, sulphamethoxazole-trimethoprim and nalidixic acid as well as new-generation antibiotics such as fluoroquinolones. Antibiotic resistance is a serious threat to patient management.

Manhiça HDSS launched a study to evaluate the trends of antibiotic resistance and molecular epidemiology of childhood *Shigella*-diarrhoea from a case-control study conducted among children under the age of five in Manhiça from December 2007 until November 2012. The study used the following methods:

- *Shigella* identification: Species identification was based on culture, colony morphology, biochemistry and serotyping.

- Antimicrobial susceptibility: All isolates were tested for antimicrobial susceptibility using the Kirby-Bauer disk diffusion method following Clinical and Laboratory Standards Institute guidelines.
- Mechanisms of resistance: PCR-based techniques were applied for detection of mechanisms of AMR.
- Genetic diversity: To evaluate whether the isolates were genetically related, pulsed-field gel electrophoresis (PFGE) was done according the PulseNet standardised protocol for typing of foodborne pathogens. A clone was defined with 85% similarity.

The study reached the following conclusions:

- There is a high diversity of *Shigella* spp causing diarrhoea in Mozambican children.
- Multidrug resistant isolates follow clonal spreading.
- The presence of MDR carriers in the community is an alert for the importance of community-based interventions for infection prevention and control.
- Continuous disease surveillance is required to track changes over time.

The proposed next steps entail virulence studies for the identification of virulence genes and clonal studies, possibly multi-locus sequence typing analysis or whole genome sequencing.

Flavonoids with Significant Antibacterial Activity from *Pseudathria hookeri* (Fabaceae) (Mr Joseph Tchamgoue, University of Yaounde I, Cameroon)

The research that is done today is for the benefit of the next generation. It is therefore surprising when people demand to see immediate results from the investment in research into new drugs.

Despite the extensive use of antibiotics and vaccination programmes, microbial infections (including bacterial, fungal, and viral infections) continue to be a leading cause of morbidity and mortality worldwide. The reasons include antibiotic resistance, the emergence of new pathogens, lack of effective new therapies and the resurgence of old pathogens.

Medicinal plants, through the variety of their secondary metabolites, can constitute a vast source for new bioactive compounds to fight against

such resistance: 25% of drugs are derived from natural products, and 61% of the 877 new chemical entity introduced between 1981 and 2002 can be traced to natural product origin. Several medicinal plants are used in folk medicine for the management of microbial infections, but few have received scientific attention to ascertain their efficiency and safety.

Pseudarthria hookeri Wight & Arn is used in folk medicine for the treatment of microbial infections including pneumonia, cough, diarrhoea and abdominal pains. It was investigated for its antibacterial activity against pathogenic bacterial strains involved in diarrhoea and respiratory infections.

The study isolated and identified the active principles, which involved collection, extraction, filtration, thin layer chromatography (TLC), purification of compounds, analysis, structure elucidation and bioassays. The antibacterial assay used the broth microdilution method: minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Acute toxicity was measured following Organisation for Economic Co-operation and Development (OECD) guidelines. The application of these methods resulted in quantification (LC-QqQ-MS).

The study reached the following conclusions:

- Chemical investigation of *P. hookeri* (Fabaceae) led to the discovery of five new compounds. Five flavonoids out of the 22 isolated compounds showed moderate to significant antibacterial activity (MIC < 10 µg/mL).
- The tested compounds showed no toxic effect on MIN-6 and 3T3 cell lines up-to 400 µM, suggesting their safety profile.
- The crude extract of the whole plant of *P. hookeri* showed no *in vivo* acute toxicity up to the highest tested dose (2 500 mg/kg).
- A rapid, sensitive and accurate method was developed for the quantification of bioactive flavonoids in *P. hookeri*. This method could be used for the quality control of herbal drugs prepared from *P. hookeri*.

An improved phytomedicine is currently being processed from the extract of this plant.

Actinobacteria Biosynthetic Potential: Bridging *in Silico* and *in Vivo* (Prof Andriy Luzhetskyy, Helmholtz Institute for Pharmaceutical Research Saarland, Germany)

In 1928, when penicillin was discovered, scientists believed that they had conquered pathogens, but Louis Pasteur (1822 – 1895) had already realised that 'the microbe always has the last word'. Soon after antibiotics were introduced to the market, drug-resistant pathogens evolved. The speed of antibiotic resistance has accelerated. A year or two after a new antibiotic is introduced to the market, resistant pathogens are now developing. The growing resistance towards established antibiotics presents a serious problem especially with infectious diseases. Pathogens that are multidrug-resistant pose a huge threat, since there are no drugs on the market to fight them.

New antibiotics can be developed through chemical synthesis or biosynthesis. Natural products have been particularly successful for the development of antibiotics. Actinobacteria have been an important source of different drugs.

There was a large gap in developing new antibiotics between 1962 and 2000. It is still possible to isolate new compounds, but there are associated problems, which discouraged industry from working on antibiotics, namely the huge range of rediscovery of known compounds. The research is very expensive, involving considerable screening, isolation and structure elucidation, and it is frustrating after all this work to keep coming up with known compounds. Industry concluded that the sources of antibiotics had been exhausted and there was nothing new to look for. This was the dominant idea for several decades until the start of the genomic era. It was realised that there was huge genetic potential through gene clusters for actinobacteria to produce many more drugs and natural products than had been discovered thus far.

Two examples of actinomycetes genome projects are:

- *S. coelicolor* A3(2): 23 secondary metabolite gene clusters are predicted (4.5% of genome).
- *S. avermitilis* ATCC31267: 30 secondary metabolite gene clusters are predicted (6.6% of genome).

Genomes were sequenced and compared, and some were found to be especially talented. *Streptomyces*, for example, has up to 50 biosynthetic gene clusters, from which 50 new natural products could be produced. Frankia is not known as a producer of antibiotics, but a number of biosynthetic gene clusters were discovered in the genome. The discovery of huge potential in the genomic era attracted industrial interest and there was huge investment.

As a small research group, the Helmholtz Institute for Pharmaceutical Research Saarland decided not to become involved in sequencing or analysing the data, as they could not compete with large role players. They decided instead to develop a genetic method for exploiting the potential.

The regulatory cascade is very complex. There has to be a signal from the environment, and then the biosynthetic gene cluster is activated through the regulatory cascade. The gene cluster will be transcribed. It is very difficult to reconstitute the conditions present in the environment in the laboratory in order to induce this expression. Another approach is thus that of synthetic biology, which entails decoupling the biosynthetic gene cluster from the environment and from the complex regulatory cascade, which is unique to every strain and could take thousands of years to understand.

Genomes contain many gene clusters that are potentially responsible for antibiotic production. The gene clusters are expressed and decoupled from the regulatory cascade. Synthetic regulatable controlling elements are taken, such as promoters, ribosome binding sites or terminators. Genetic clusters are then engineered, and brought into a chassis or host where they will be expressed and new compounds can be isolated. The following three components are required for this process:

- Synthetic controlling elements, which are predictable and not dependent on any controlling signal from the environment, and which guarantee the expression of particular gene clusters in the host.
- Heterologous host to which the biosynthetic gene cluster is brought (of which there are now several thousand).
- Actinobacteria genome mining.

Some bacteria behave in a domesticated way and are easy to work with, while others are difficult to handle. There was an idea to move from domesticated to engineered bacteria that can be more readily controlled. After looking through the whole *Streptomyces* collection, *S. albus* was found to be suitable for domestication. It has a small genome and produces a variety of different natural products, which means that it has all the precursors that are necessary on which to synthesise natural products or antibiotics.

Work began on *S. albus*. The first step was simple and straightforward. A biosynthetic gene cluster will consume the precursors (e.g. amino acids). By combining the simple precursors, an antibiotic can be built. There are many different ways to successfully clone the gene cluster.

About 20 000 natural products have been discovered from actinobacteria, but only about 1 000 gene clusters have been characterised.

Refactoring of the gene cluster with synthetic controlling elements and bringing this into the host will yield the new compound. Many new compounds have been discovered, which will hopefully lead to the discovery of active compounds against MDR bacteria.

Discussion

Question (Dr Patrick Arthur): Are you willing to go to the next level and teach the new clusters new tricks?

Response (Prof Luzhetskyy): Through biosynthesis, the specificity of the enzyme can be changed. Genes can be combined from different gene clusters in order to create new chemistry.

Question (Prof Sabiha Essack): Has there ever been an outbreak of diarrhoea due to *Shingella isolates*?

Response (Mr Delfino Vubil): The research did not go into detail in looking at the period to see if there was an outbreak.

Question: Have you considered using tRNA as a chassis for all genes?

Response (Prof Luzhetskyy): *S. albus* was explored first because of its large variety of biosynthetic gene clusters, but in principle it would be possible to try to adapt to other clusters from different organisms.

Question (Dr Patrick Arthur): Is it possible to take clusters from fungi into *Streptomyces*?

Response (Prof Luzhetskyy): This would be difficult. It would involve re-synthesis and would entail laborious work with a low rate of success. It would be better to develop new hosts based on fungi.

SESSION 3: DINNER

Facilitator: Prof Quarraisha Abdool Karim, Associate Scientific Director, Centre for the AIDS Programme of Research in South Africa (CAPRISA)

Prof Karim opened the event and welcomed everyone. She acknowledged funding for the event from ASSAf, Leopoldina, ICSU ROA and the South African Department of Science and Technology (DST). ASSAf and Leopoldina were celebrating five years of successful collaboration and had just signed another MoU for a further five years.

Keynote Address IV: Building Research Capacity and Transformation in Health Research (Prof Glenda Gray, President and Chief Executive Officer (CEO), Medical Research Council, South Africa (MRCSA))

State of science in Africa and rationale for investing in science

The South African Medical Research Council (SAMRC) is the custodian for medical research in South Africa and the biggest funder (and government funder) of medical science in Africa. Scientific excellence is central to everything that the SAMRC does, and the science that it funds is always internationally peer-reviewed and competitive. The SAMRC funds research based on local development priorities and the ten most common causes of mortality in the country, given the funding limitations. The research also looks at disability and quality of life. The SAMRC focuses its efforts on developing scientific capacity and transforming the pipeline of researchers in South Africa.

There is a problem of lack of local investment in research. South Africa spends only 0.9% of its gross domestic product (GDP) on research and development (R&D). Only 1% of global investment in research and development (R&D) is spent in Africa, and only 0.1% of world patents are held in Africa.

Scientific productivity is a much better predictor of economic wealth and human development than any other variables. Africa has a lack of critical mass among researchers. In Africa there are only 198 researchers per million inhabitants, compared to over 4 000 researchers per million inhabitants in the UK or USA. It is alarming that 20 000 professionals leave Africa per annum to high-income countries. It is a challenge to try to keep professionals and researchers in African countries.

The unequal contribution and participation in science in Africa needs to be addressed and changed. The collaboration between ASSAf and Germany, as well as ASSAf and Uganda, is important in the efforts to proliferate scientific research in Africa. Africa has 12% of the global population, but produces less than 1% of total science output. The increase in research in Africa between 2003 and 2012 was driven by health science research (4% annual growth), which represents 45% of all research in sub-Saharan Africa. Africa has a disproportionately large share of global AIDS deaths. If important diseases are to be addressed, science will have to grow on the African continent.

The SAMRC is committed to changing this picture by:
- Administering health research effectively and efficiently.
- Leading the generation of new knowledge.
- Supporting innovation and technology development to improve health.
- Building capacity for the long-term sustainability of the country's health research.

There is no reason why South Africa should not lead the world in many areas of health research, and the country is not short of problems to solve or lacking in innovation. South Africa has a quadruple burden of disease through four colliding epidemics:
- Maternal, new-born and child health.
- HIV/AIDS and TB.
- Non-communicable diseases.
- Violence and injury.

Scientific research leads to new knowledge, technologies and clinical treatment along the spectrum from basic science, to the development of new paradigms, and the impact of translational science leading to products, discoveries and innovations that will have a direct impact on society.

Addressing capacity development in health sciences research

Addressing capacity development in health sciences research in South Africa entails:
- Building capacity for the long-term sustainability of the country's health research.
- Capacity development in health research.
- Addressing succession and sustainability.

- Training clinical scientists to respond to South Africa's health needs.
- Developing highly skilled researchers for global competitiveness.

The ASSAf consensus study of clinical medicine and clinical research in 2011 identified the paucity of clinician scientists (MD PhDs). This needed to be addressed by increasing the number of PhD-holders involved in clinical research. The SAMRC therefore moved from a focus on funding Masters to funding doctoral programmes, with a target of funding 100 health science-related PhDs per annum. There has been gradual progress towards this target, with 61 PhDs funded in 2016/17 (an increase from 16 in 2011/12).

The SAMRC has also invested in building the next generation of black scientists and addressing the discrepancies in transformation. South Africa now has a vibrant group of future black scientists.

Gender and science have been another challenge in order to keep women in science, ensure that they are valued and address the issues that cause attrition, which include:

- A one-way career flow with no interruptions does not reflect the lived experience of women.
- Senior women are often more vulnerable in their positions than men.
- Many women choose technical careers rather than academic or managerial careers.
- Women may have to move backwards in their career to accommodate their family.
- Women often say they have to out-perform men to advance and be taken seriously.
- Leaks of women from scientific careers correspond to family formation but may not be chosen freely. Women face long hours at work, are required to be mobile, and the demands of the career of a spouse are often incompatible with family responsibilities.

Unless there is a critical mass of women or black scientists, the gender and racial stereotypes are reinforced, namely that good scientists tend to be white men. This is a particular challenge for South Africa. Science often has a very masculine and very Western model of success characterised by long working hours, uninterrupted scientific career and active participation in extracurricular activities, such as advisory boards and panels.

The SAMRC is committed to gender transformation and supporting women to become science leaders. In 2018, three-quarters of the SAMRC's capacity development grants were awarded to women, and half to black African scientists. There has also been transformation in self-initiated research grants. Two streams of research grants were created (one for early-stage investigators and one for mid-career and advanced investigators), so that like could compete with like. The variables of race, gender and graduating from a historically disadvantaged institution were weighted. These interventions helped to open the field for transformation. By 2018, 41% of SAMRC grants were awarded to African researchers, compared to 11% in 2012. All the SAMRC's grants are internationally peer-reviewed following an NIH review process.

The SAMRC began an intervention to build mid-career scientists and mentor them to becoming science leaders in the interests of succession planning. The programme is currently supporting five mid-career medical scientists with a focus on clinical researchers and women.

The SAMRC is committed to supporting clinical research among early to mid-career scientists. The South African Clinician Scientists' Society would be launched on 11 May 2018 with the following objects:

- To create a platform for clinicians to develop as scientists.
- To establish an environment for clinician scientists to discuss research ideas and collaboration.
- To liaise with national and international stakeholders.

This society would be the first of its kind. It is the initiative of Dr Salome Maswime, a young and committed SAMRC fellow.

In order to address the shortage of support for postdoctoral internships, the SAMRC created programmes for the development of research skills, including:

- NCD basic science support through the relationship between the University of Zululand and SAMRC supporting 30 interns.
- Intramural postdoctoral interns.
- Extramural postdoctoral interns.

The DST-NRF-SAMRC South African Research Chairs Initiative (SARChI) Chair in Biostatistics was recently launched with co-funding for five years to address the paucity of biostatisticians in South Africa. The chair is aimed at developing postdoctoral fellows, doctoral, Masters and honours students.

In 2015, the SAMRC launched a programme to strengthen institutional research capacity at eight historically disadvantaged institutions with the following objectives:

- To strengthen research capacity at selected universities.
- To leverage funding for research projects and programmes (collaborative), and for essential infrastructure to conduct research.
- To create an enabling research environment.
- To gear the universities for self-sustainability.
- To initiate, maintain and grow a robust and sustainable research culture.

Each university receives R1 million per annum for five years. There are 18 investigators at these universities, with more than 20 research projects, and several Masters and doctoral candidates. The first papers were published in 2016 within a year of the start of the programme, and the first cohort of graduates graduated in 2017. This programme has been particularly successful because of the mentoring to enable investigators to become successful and competitive.

The SAMRC has developed international collaboration to improve health outcomes in South Africa. The idea was to develop collaborative networks, improve grant-writing capacity, conduct world-class research and produce world-class scientists. The most ambitious of these programmes was SAMRC-NIH collaboration, which brought together USA and South African researchers at a joint Summit on HIV/AIDS, TB and HIV related Malignancies, held in Durban on 17 – 18 June 2013. Each country dedicated US$40 million to the programme, which has been very fruitful and is about to be renewed. In the next call, collaboration with other African scientists will be introduced.

Another exciting initiative was the launch of the Whole Genome Sequencing Research Institute in South Africa in 2018, in collaboration with the Beijing Genome Institute. The institute is developing the field of bioinformatics at the country level.

Research capacity enhancement and sustainability require a multi-factorial approach with engagement in all the following areas in order to be successful:

- Building research capacity along the whole value chain of individuals, departments and institutions.
- Improving, refining and growing research expertise and portfolios by identifying research niches that fit into the national burden of disease but are also globally relevant.
- Forming collaborative research networks and partnerships.
- Establishing independent researchers, departments and institutions, including successful grantsmanship from diverse sources to fund research and attract new partnerships.
- Realising the development of skilled researchers, globally competitive departments and excellent institutions and leveraging research capacity for research translation contributing to the knowledge economy and GDP.

Science has the power to save lives, particularly in South Africa.

Prof Kaufmann thanked Dr Gray on behalf of colleagues at Leopoldina, the UNAS and ASSAf.

SESSION 4

Facilitator: Dr Oladoyin Odubanjo, Nigerian Academy of Science, Nigeria

Keynote Address V: Novel Biomarkers and Vaccines for Tuberculosis Control (Prof Stefan Kaufmann, Director, Max Plank Institute for Infection Biology, Germany)

In pursuing the Millennium Development Goals and later the Sustainable Development Goals, the UN decided to convene high-level meetings on health and other issues related to the goals in order for partners to take responsibility. Four meetings have thus far taken place on health issues:
2011: High-level meeting on non-communicable diseases.
2014: High-level meeting on Ebola.
2016: High-level meeting on ending AIDS.
2016: High-level meeting on AMR.

The fifth high-level meeting on tuberculosis is scheduled for 26 September 2018 in New York. This meeting was preceded by pre-meetings: the Global TB Ministerial Conference in Moscow in November 2017, and the STOP TB Partnership Board meeting in Delhi in March 2018. This is intended to encourage global commitment to end TB as the leading infectious killer and one of the top ten causes of death worldwide. Annually, 10.4 million people fall ill with TB, 1.8 million die of TB, and 0.5 million develop multidrug-resistant TB. Multi-sectoral action is required to accelerate global and national TB responses, involving the health, finance, labour, justice and social development sectors, and all stakeholders including government, civil society, NGOs, the private sector and academia. New intervention methods are needed. The presentation addresses two interventions: TB biomarkers that measure host responses rather than the epidemiological agent, and a new TB vaccine.

The immunopathology of TB follows several stages:

- If a TB-infected person coughs, there is a strong likelihood of infecting other people, because the pathogen is transmitted by airborne particles.
- The bacteria enter the alveoli, where they are engulfed by monocytes and granulocytes, but most importantly also by the dendritic cells in the epithelial layer, which transport the bacterial load into the lymph nodes.

- The T cells are activated, and after several weeks CD4 T cells and CDA T cells contribute to the development of a lesion, known as a sold granuloma, in which the bacteria are contained. These lesions often last life long, affecting between 1.7 and 2 billion people in the world who have latent TB infection but are healthy. Sometimes the lesions disappear, but the bacteria stay elsewhere; for example, the bone marrow is thought to be a major reservoir.
- After a year or even several decades, in 5 – 10% of individuals, a cancerous lesion develops, a caseating granuloma (first necrotic caseating and then cavitory) develops and cavities are formed, and the bacteria multiply and may be spread to other organs, and to other individuals through contagious disease.

TB biomarkers could be a diagnostic tool for the future, even at the point of care. A study was launched with funding from the Gates Foundation to investigate host biomarkers in TB and compare the differences in the host response between people exposed to TB who never become sick (1.7 billion) and those who develop serious disease (>10.4 million 2016). The study looked at blood cell transcriptomics (i.e. gene expression profiles). The presentation focuses on transcripts, and metabolites are only mentioned.

Biomarkers are very successful for diagnosing TB, with biosignature with high sensitivity and specificity for differential diagnosis between active TB and latent tuberculosis infection. Biosignature is then used with a predictive marker for the disease prognosis (i.e. how soon a person with latent TB infection will develop active TB).

The process can be simplified. Only three to four biomarkers are required to reliably diagnose TB. The top markers for tailored diagnostic signature in a peripheral blood mononuclear cell are:

- FcgR I, an antibody Fc binding receptor.
- Lactoferrin, which is involved in iron uptake.
- Guanylate-binding proteins, which are induced by type I and type II events.

This combination has been shown to work well, but other combinations can be used. This approach was used with 85% specificity and 85% sensitivity in The Gambia, Uganda and South Africa under the Grand Challenges in Global Health research initiative launched by the Bill and Melinda Gates Foundation. It was then validated in Bangalore, India and Eastern Europe

with the same results. Different combinations of three to four markers combined in decision trees also work well.

Having solved TB diagnosis, the core activity of the Grand Challenge programme is now to investigate TB prognosis, which Prof Kaufmann headed as the principal investigator until 2013. It was then decided to move the research to South Africa, with Stellenbosch University and the University of Cape Town as major players.

The index case involved 1 098 newly diagnosed TB patients at seven sites across Africa, and 4 462 household contacts. Household contacts that are willing to participate in the study are followed over two years. Blood is taken immediately after exposure to TB, which is at approximately the time of diagnosis of the index case, and six, 18 and 24 months later. At the end of the two-year period, 2.2% of household contact individuals developed TB, and 97.8% remained healthy. The rate of infection was lower than the expected 4 – 5%, which is the average in South Africa. With over 80% sensitivity and specificity, it is possible to predict which individuals with latent TB infection will develop TB disease within the next 12 to 24 months using a transcript-based GC6 biosignature.

It is possible to further minimise the risk by introducing pair ratios (upregulated marker/downregulated marker), so that two pairs of two selected biomarkers can predict TB in latently infected individuals. There is the potential to develop a PCR test using just a few biomarkers for point-of-care prognosis of TB.

This work was based on transcripts. It was hoped that a dipstick could be developed in order to use metabolites for diagnosis. This proved to be difficult, but it does work, although with less sensitivity and specificity in the range of 70 – 80%.

Metabolomic and transcriptomic signatures can be harnessed for both diagnosis and prognosis of TB. Prognostic signatures in reality detect disease progression six to 12 months prior to active disease. The original signature used 16 transcripts, and the decision trees and pair ratios have now been reduced to two marker pairs (i.e. four markers). Multiplatforms are now being analysed for further optimisation of the system and yield higher sensitivity and specificity.

The issue is what we are really looking at. It was realised that another group of individuals needed to be defined, namely those with subclinical disease. These people are healthy, but the markers are so sensitive that light inflammation can be detected. Since most of those with subclinical TB will eventually develop active TB, TB prognosis can be determined by diagnosing subclinical TB. This is relevant for preventive therapy before the onset of the clinical disease and transmission. The University of Cape Town is testing whether identifying subclinical individuals and offering them preventive therapy can succeed in preventing active TB and transmission of the disease. This would involve drugs that prevent transmission. Diagnosing subclinical individuals could be used for stratification for clinical trials.

It might be asked why there is a need for a new TB vaccine, since BCG is available. BCG protects against extrapulmonary TB in neonates, but poorly against pulmonary TB in any age group, notably in developing countries with the highest TB incidences. Better vaccines are therefore urgently needed.

It was decided to use BCG as a platform and improve it using Vakzine Projekt Management (VPM) 1002, the most advanced live TB vaccine candidate. Next-generation BCG has been produced, improved by genetic modification. This vaccine has now reached phase II and phase III trials as a pre-exposure BCG replacement for neonates. It will be tested for pre and post-exposure *Mtb* as a BCG boost for adults, and as a post-exposure *Mtb* for prevention of recurrence. The vaccine will be used for prevention of infection, prevention of disease and prevention of recurrence. This is quite ambitious.

The new vaccine was developed by introducing the gene for listeriolysin not as an antigen but as the molecule that perforates the phagosomal membrane. When the listeriolysin perforates the membrane, proline antigens form the recombinant vaccine, egress into the cytosol, and CD4 and CD8 T cells are stimulated (whereas BCG primarily stimulates CD4 cells). Perforation also allows egress of listeriolysin and cathepsins inducing apoptosis, which leads to the release of vesicles that contain mycobacterial components. Cross-priming, which is much better than primary priming, is induced, and a broad spectrum of CD8 T cells, Th17 cells, inflammatory CD4 cells, Th1 cells, central memory T cells and helper cells are induced. Ultimately, autophagy and inflammation are induced, because double-stranded DNA is released, which is sensed by the STING

(stimulator of interferon genes) molecule, resulting in autophagy or xenophagy and much stronger immunity.

The vaccine has been shown to be efficient and safe in preclinical studies. P1-level was approved Large-scale GMP production (fermentation) has been achieved. Safety and toxicity have been approved. The hygromycin resistance marker has been removed. The vaccine was licensed worldwide to VPM and has been renamed VPM1002. Recently it was sub-licensed to the Serum Institute of India, the largest vaccine producer in terms of doses, which is continuing with the trials of the vaccine.

The vaccine has undergone the following clinical trials:

- Phase I trial in Germany NCT 00749034: successfully completed in 2009; three escalating doses were given to young adults (104, 105, 106 rBCGΔureC::hly, comparator 106 BCG).
- Phase I trial in South Africa NCT 01113281: successfully completed in 2011; three escalating doses given to young adults (104, 105, 106 rBCGΔureC::hly, comparator 106 BCG).
- Phase IIa trial in South Africa NCT 01479972: successfully completed in 2013; three escalating doses given to newborns (104, 105, 106 rBCGΔureC::hly, comparator 106 BCG).
- Phase II trial in South Africa NCT02391415: 416 HIV-exposed/unexposed neonates; the first baby was vaccinated on 11 June 2015; recruitment was completed in September 2016, and unblinding was expected late in 2018.
- Multicentric phase I/II therapy trial for bladder cancer NCT02371447 in Switzerland: first instillation on 21 September 2015; phase I had been completed, and phase II had started.

The issue was then to design a phase III clinical trial with limited financial resources. If one in 100 people are assumed to develop TB, and 200 cases are required in the control group for statistical reasons, a minimum of 20 000 participants are required per group, and hence 40 000 participants in total over five years. A clinical trial of this magnitude would cost US$200 million.

TB vaccine trial design could be slightly improved by using household contacts. With this approach, approximately 6 000 participants would be required per group, totalling 12 000 over three years and costing US$36 million. This approach would be followed from 2019. The Indian Council of Medical Research had decided to test the efficacy of the VPM1002

vaccine in a household context study together with a competitor. The study had been submitted and the results were expected in 2023. This would involve both pre and post-exposure individuals. Most of the household contacts (an estimated 90%) would already be infected.

Another way to improve TB vaccine trial design would be through stratification for TB high risk. This study would comprise neonates, since about five in 100 develop TB disease despite BCG vaccination. This would involve 4 000 participants per group, with a total of 8 000 over two years, costing US$16 million. A phase III multicentre efficacy trial was being prepared in HIV-exposed/unexposed neonates in sub-Saharan Africa. BCG is not endorsed for HIV-exposed neonates. The trial is expected to start in the third or fourth quarter of 2018, funded by the EDCTP, and the results are expected by 2024. The objective of the trial would be pre-exposure BCG replacement, with the end points of safety and efficacy against infection and prevention of disease over BCG.

Prevention of recurrence was another area of focus in improving TB vaccine trial design. In India, about 10% of those who have been cured using TB drugs will develop TB again after one year, whether through reinfection (30 – 40%) or relapse (60 – 70%). A trial of this group would require only about 2 000 participants, totalling 4 000 over one year, at a cost of US$4 million. The trial on the prevention of recurrence began in 2018. The first vaccine was administered on 5 January 2018, and the trial will be concluded by the end of 2019, with unblinding in 2020. This group is highly susceptible.

Another way of designing a clinical trial would be to recruit people with subclinical TB (i.e. those predicted to develop the disease within a year). This would require 300 participants per group, totalling 600 over one year, at a cost of US$0.6 million.

WHO has set the ambitious target of reducing morbidity by 90% and mortality by 95% by 2030 – 2035. Now that biomarkers are available to identify and introduce individuals into preventive treatment, and with a new TB vaccine on the horizon, the goal is starting to become somewhat more realistic.

Discussion

Question: If subjects are reliably identified at subclinical level before they develop the active disease, could the vaccine be administered to stop the disease?

Response (Prof Kaufmann): There were concerted efforts to find a few metabolically stable markers. Individuals have to be screened each year. A surveillance study can be conducted in populations at high risk to identify those who are likely to develop TB in the next year and offer treatment. This is a very expensive approach. A study of this nature is being conducted at the University of Cape Town with funding from the Gates Foundation, the data from which will soon become available. Four biomarkers are now used for a simple low-cost assay.

Question (Dr Norbert Heinrich): It was mentioned that there is specificity of 80% one year before diagnosis with TB, and that this increases in the six months before diagnosis. What does this indicate about the incubation period, and when does the infection event actually occur?

Response (Prof Kaufmann): This is not known. Investigating this would involve non-human primate studies such as those conducted by Flynn in the USA. In Prof Kaufmann's study, all individuals who developed TB within three months of the index case diagnosis were discarded. Individuals are infected by the index case and give out latent TB; fewer than 3% continue to direct TB. TB has the highest rate of infection in the first year two years. The literature reported that 5% continued to direct TB, but Prof Kaufmann's study found this to be much lower, and recruitment continued up to 100 cases. The initial goal was to recruit 3 500 participants, but this rose to 4 500 in order to get 100 cases. Prof Kaufmann's research was a complex pan-African study with better bioinformatics.

Keynote Address VI: Virulence Factors of the Human-Pathogenic Fungus *Aspergillus fumigatus* and Novel Antibiotics (Prof Axel Brakhage, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Germany)

Germany has four non-university research institutes, including the Max Planck Society, the Helmholtz Association of German Research Centres and the Leibniz Association, which comprises 90 institutes in five sections, with 17 200 employees and an annual budget of €1.5 billion, half of which comes from the federal government and half from federal states.

The Leibniz Institute for Natural Product Research and Infection Biology has 440 employees, including ten professors, 130 doctoral researchers, 150 students and ten start-ups that emerged from the institute. The institute works very closely with universities, to the extent that it is almost part of the university system.

The motivation of the institute is that its research must have relevance for society. Its research focuses on multi-resistant pathogens and human-pathogenic fungi that cause high mortality because diagnosis tends to be unspecific and is often too late, and therapy is ineffective. New anti-infectives are needed. Another aspect of the research is active compounds in natural products, which form the basis for many pharmaceuticals. The pipelines for new anti-infectives are empty, pointing to a major societal need.

Fungi infect billions of people every year, including:

- Superficial infections of the skin, mucosal surfaces and nails. About a quarter of the world's population is infected with dermatophytes. Ninety per cent of HIV-infected patients are infected with oral *Candida*.
- Life-threatening fungal infections. Fungi have become a major problem in the clinical setting and kill at least as many people as tuberculosis or malaria.

Significant invasive fungal infections include:

- Aspergillosis (*Aspergillus fumigatus*), which accounts for over 200 000 infections per year, with 30 – 95% mortality rates in infected populations. Invasive aspergillosis infections are often associated with TB because of the defect of the lungs.

- Candidiasis (*Candida albicans*), which accounts for over 400 000 infections per year, with 46 – 75% mortality rates in infected populations.
- Cryptococcosis (*Cryptococcus neoformans*), which accounts for over one million infections per year, with 20 – 70% mortality rates in infected populations. Cryptococcosis occurs particularly in sub-Saharan Africa, often associated with HIV.

The mortality rates for these infections are extremely high.

Prof Brakhage works on *A. fumigatus*, a common fungus. People inhale the spores, and can become infected, especially immune-suppressed patients, with mortality rates of up to 90% because it is very difficult to treat. Prof Brakhage's laboratory is particularly interested in the interaction between the fungus and immune cells. When conidia are inhaled, many immune cells are involved in preventing infection, but the fungus has immune-evasion mechanisms. Two such mechanisms have been discovered involving macrophages. The fungus has surface molecules, which are the first contact with the immune system. Two surface molecules were mentioned:

- Rodlet layer: Spores have distinctive proteins known as rodlets, which are like a shell for the spore to protect it against adverse environmental conditions. The rodlet layer consists of a protein called Hydrophobin RodA, which prevents immune recognition of airborne fungal spores by humans. It is difficult to isolate this protein, requiring very harsh conditions, and only resting conidia are found, not swollen or germinating conidia.
- Dihydroxy naphthalene (DHN) melanin: A gene cluster was discovered, and when the genes were deleted, white conidia were obtained. When these conidia are introduced to mice, they are avirulent (i.e. they have lost their virulence). It was found that the pigment is important for virulence and is similar to mycobacteria, in that the melanin inhibits the acidification of fungal lysosomes. If the pigment is missing, the phagosome fuses with the lysosome and kills the conidia. This work has not yet been published.

The mechanism of DHN-melanin interference with macrophages was investigated. Membranes form lipid rafts, which are an accumulation of cholesterol, glycosphingolipids, and proteins that keep the lipid raft together. The lipid rafts are essential for receptors to be positioned in a membrane, and are important for signalling processes. Melanin interferes with the formation of lipid rafts in macrophages. The phagolysosomal

membrane surrounding conidia showed a reduced assembled vATPase when they had melanin.

The three-step mechanism works as follows:

- Conidial proteins mask immunogenic structures and bind human immune regulatory proteins.
- DHN-melanin prevents apoptosis.
- DHN-melanin prevents full acidification of the phagolysosome by interference with lipid rafts.

Recognition of DHN-melanin by a C-type lectin receptor is required for immunity to *Aspergillus*.

Discovery of novel antibiotics

The focus would be on natural products and antibiotics from fungi. Fungi are chemical factories. It is estimated that they produce millions of compounds, most of which are not yet known. Penicillin, cyclosporine and statins, for example, were derived from fungi.

The biological problem of natural product research is that microorganisms do not use their full genetic potential to produce natural products under laboratory conditions; they need the ecological context to activate the gene clusters because ecological triggers are required. The challenge was to find a way of activating the gene clusters.

Natural products can be discovered through genome mining. In one example of a silent gene cluster in the genome of *Aspergillus nidulans*, a regulatory gene that formed part of the gene cluster was fused with an inducible promoter and put back into the fungus. When the regulatory gene was activated, all the genes of the cluster were activated. New peaks appeared. It was reasoned that new compounds were formed, which were then isolated, and the structures were elucidated. Through synthetic biology or genetic engineering, there is now access to many silent gene clusters.

Researchers are interested in the ecological conditions that activate gene clusters to produce antibiotics. It is believed that natural products are the chemical language of the microorganism. In order to find such compounds, the *Aspergillus nidulans* fungus was co-cultivated with 50

different actinomycetes that live in the soil. All the gene clusters in the fungus were analysed for activation of silent gene clusters. A single bacterial strain, *S. rapamycinicus*, activated a silent fungal gene cluster. Activation of the *ors* gene cluster requires intimate physical contact between *A. nidulans* and *S. rapamycinicus*.

Jagaricin was presented as an example of using the ecological context to discover new compounds. Jagaricin, a novel antifungal compound, was developed by Dr Christian Hertweck's group. Jagaricin is produced by a bacterium that infects mushrooms such as white button. The compound belongs to the group of lipopeptides. Apart from aiding in the infestation of the mushroom, it is active against a variety of other fungi including human pathogens such as *A. fumigatus* and *C. albicans*.

The investigators became aware of less-investigated bacteria that can infect mushrooms and assumed that the bacteria probably employ small compounds that weaken or kill the fungus. They reasoned that such compounds would be potentially active against other fungi and thus perhaps also useful for treating mycoses.

Sequencing the genome of the bacterium proved to be very helpful because with the genomic inventory at hand they were able to predict its potential for metabolite production. They noted that the candidate genes were silent under standardised culture conditions in the laboratory. Thus, they wanted to catch the bacterium in action and monitor how it infects the mushroom.

Using a modern method to visualise the production of metabolites (imaging mass spectrometry), they discovered that a compound with a particular molecular mass accumulates in lesions of the mushroom tissue. They succeeded in finding growth conditions to produce sufficient amounts of the antifungal agent for a full structural elucidation and biological testing.

BTZ043 (Benzothiazinone) is a new drug against tuberculosis. It is extremely active against *Mycobacterium tuberculosis*. The structure was isolated, the compound was synthesised and a patent was filed. A consortium comprising experts from the Leibniz Institute for Natural Product Research and Infection Biology and other specialists in the field discovered that Benzothiazinones kill *Mycobacterium tuberculosis* by blocking arabinan

synthesis. Inhibition of this enzymatic activity abolishes the formation of decaprenylphosphoryl arabinose, a key precursor required for the synthesis of the cell-wall arabinans, thus provoking cell lysis and bacterial death.

Industry is no longer willing to invest in development research to the extent that they did in the past. Academia needs structures in order to fill the gap between research and industry. Fortunately, InfectControl 2020 was prepared to support the further development of the compounds. InfectControl 2020 is a consortium of representatives from enterprises and academia that jointly aims at developing solutions regarding these problems on a national and global level. It is funded by a grant from the Federal Ministry of Education and Research in Germany. The Leibniz Institute for Natural Product Research and Infection Biology together with the Friedrich Löffler Institute developed a transfer group for anti-infectives with the role of filling the gap between research and industry by managing novel compounds, developing novel animal models, preclinical trials and supporting clinical trials of the clinical partner, Prof M Hölscher at the University of Munich.

The clinical trials of the BTZ043 compound have been completed, and funding is available to enter the preclinical phase once the work has been approved. All this work has been publicly-funded; €14 million were raised.

Discussion

Question (Dr Collen Masimirembwa): What is the failure rate of antibiotics in these programmes? In most drug discovery programmes, the attrition rates are high. What gives you optimism on the basis of preclinical data that these new antibiotics will succeed?

Response (Prof Axel Brakhage): The institute isolates 50 new classes of compounds per year and most are excluded. Only when a compound is found that is very effective *in vivo* in animal models with low toxicity is there consideration of taking it further. The metabolites that are derived in the bloodstream also have to be analysed. The Leibniz Institute for Natural Product Research and Infection has specialists in the compounds for this type of work, but there are very few such experts throughout the world. The success rate is higher if these issues are addressed by the same team from discovery right through to the eventual development of the drug.

Keynote Address VII: Refocusing on STIs (Prof Koleka Mlisana, University of KwaZulu-Natal, South Africa)

The way in which sexually transmitted infections (STIs) have been managed is altogether inadequate. Prof Mlisana would like to see the South African government change this.

Epidemiology and HIV risk

The complications of STIs include:

- Infertility (male and female): In sub-Saharan Africa, untreated genital infections are responsible for 85% of infertility among women seeking infertility care.
- Pelvic inflammatory disease (PID) in women.
- Urinary tract complications.
- Cervical cancer: human papillomavirus (HPV) infection causes 530 000 cases of cervical cancer and 275 000 cervical cancer deaths each year.
- Pregnancy-related morbidities such as ectopic pregnancies and pre-term labour.
- HIV transmission and acquisition: If these are to be reduced, STIs cannot be ignored.
- Genital inflammation.
- Psychological impact.

The causative agents of the most common STIs include viruses and bacteria. Common viral STIs include warts, herpes, hepatitis and AIDS, and bacterial STIs include chlamydia, gonorrhoea and syphilis.

More than one million STIs are acquired every day worldwide, including an estimated 357 million new infections with one of the following four STIs each year: chlamydia, gonorrhoea, syphilis and trichomoniasis. More than 500 million people are estimated to have genital infection with herpes simplex virus (HSV). More than 290 million women have HPV infection. There are high prevalence rates in Africa, particularly chlamydia and trichomoniasis. In South Africa, there are chlamydia and trichomoniasis prevalence rate of 10 – 18%.

A person with one STI is more likely to become infected with another one. A study in South Africa by D Moodley *et al.* found a high rate of STIs

among pregnant women, including both those with HIV and those who were uninfected with HIV.

STIs, bacterial vaginosis and HIV risk

- The vaginal environment greatly alters the likelihood of HIV transmission.
- The presence of STIs, epithelial disruptions and any factor that increases inflammatory cytokines increases the likelihood of HIV transmission.
- In women, the acidification of the vagina by lactic acid from lactobacilli provides protection from vaginal infections.
- BV or the absence of vaginal lactobacilli is an independent risk factor for STIs such as HIV, HPV and HSV infections.
- Women with bacterial vaginosis (BV) as well as men whose female partners have BV have a more than two-fold higher risk of transmitting HIV.
- Most STIs are curable (especially bacterial STIs), but if untreated lead to significant morbidities such as ectopic pregnancies, infertility, foetal and neonatal complications.

Lactobacillus species as biomarkers and agents can promote various aspects of vaginal health. A healthy 'normal' vaginal environment is dominated by *Lactobacillus* species with a pH of less than 4.5. This description may not be perfectly applicable to women of African descent, who have diverse microbial communities. Loss of *Lactobacillus* may lead to bacterial vaginosis, a microbial dysbiosis, rather than an STI.

Bacterial vaginosis occurs in over 50% of sexually active women and is associated with increased risk for HIV transmission or acquisition. Bacterial vaginosis is also associated with reproductive and obstetric sequelae, including PID, spontaneous abortions and pre-term labour. As many as 75% of women may be asymptomatic.

Current management of STIs

Like most poorly resourced countries, South Africa uses syndromic management of STIs, because STIs signs and symptoms are rarely specific to a particular causative agent due to dual and multiple infections. Most STI organisms are fastidious and difficult to culture for susceptibility testing. Laboratories for diagnosis are either non-existent or non-functional due to lack of resources. Dual infections are quite common, and both clinician and laboratory may miss one of them. In poorly resourced

circumstances, there are benefits to treating patients syndromically, in that it is not necessary to wait for laboratory results; patient management is improved through treatment at first contact; and this approach allows for the decentralisation of the management of STIs to primary health care levels. Syndromic management thus makes sense, but is only possible in patients who are symptomatic.

A study in Kenya evaluated syndromic management of STIs in the Kisumu incidence cohort study. The findings suggest that syndromic management of STIs is not a sufficient tool for STI diagnosis in this setting; development and improvement of STI diagnostic capabilities through laboratory confirmation is needed in resource-limited settings.

Prof Mlisana and her group conducted a study to follow women in an acute infection cohort study. Any of the participants with clinical symptoms were treated syndromically, and all the participants were tested for STIs every six months. Women with symptomatic diagnosis of STIs were compared with the laboratory diagnosis of STIs. The result was 12.3% sensitivity, which means that almost 87.7% of patients were missed who had laboratory-diagnosed STIs but no clinical symptoms. Among those who were treated syndromically (e.g. because they had a vaginal discharge, which could be due to causes other than STIs), 65.8% did not have STIs according to the laboratory results. Current syndromic management thus not only under-diagnoses, but also results in over-treating. There is a place for syndromic management for patients who are symptomatic, but there is a need to look beyond this approach.

Asymptomatic infections and genital inflammation

A study compared cytokine levels and genital inflammation in women with and without symptoms of STIs. It was found that cytokines were increased in both symptomatic and asymptomatic women. This means that asymptomatic women also had inflammation and were thus susceptible to the risk of HIV infection. Another study found that biological susceptibility in the female genital tract alters the protective efficacy of tenofovir gel.

Genital inflammation in women increases the risk of HIV transmission and acquisition, and this is seen in both symptomatic and asymptomatic infections. Genital inflammation may be caused by factors other than STIs, such as vaginal douching. The findings undermine the effectiveness

of tenofovir gel in preventing HIV acquisition in women, especially for those with genital inflammation. Genital inflammation therefore needs to be managed.

Screening is an important approach for identifying and treating infected individuals with asymptomatic STIs who would otherwise go undetected, but routine screening for all potential STIs in all patients is cost-prohibitive. Targeted screening of asymptomatic patients in specified risk groups is more feasible. Screening for chlamydia has been the most extensively studied. The benefits of screening are to reduce the personal risk of reproductive sequelae in women, to reduce the likelihood of reinfection of sex partners, and to reduce overall transmission of these infections in men.

For the purposes of targeted screening, the risk for STIs can be assessed on the basis of behavioural risk factors, including:

- New sex partner in past 60 days.
- Multiple sex partners or sex partner with multiple concurrent sex partners.
- No or inconsistent condom use when not in a mutually monogamous sexual partnership.
- Trading sex for money or drugs.
- Sexual contact (oral, anal, penile or vaginal) with sex workers.
- Meeting anonymous partners on the internet.

Risk groups associated with high STI prevalence include:

- Young age (15 to 24 years old).
- Men who have sex with men.
- History of a prior STI.
- Unmarried status.
- Lower socioeconomic status, or high-school education or less.
- Admission to correctional facility or juvenile detention centre.
- Illicit drug use.

Better diagnostics are available for targeted screening of high-risk groups. Pregnant women have been screened for syphilis for a long time. It is now possible to screen for hepatitis B or hepatitis C. Nucleic acid amplification tests are available for screening for *N. gonorrhoeae*, *C. trachomatis* and *T. vaginalis* using less invasive samples such as urine, especially men. There is enough evidence to show that self-collected vaginal swabs give reliable results.

Resistance in gonococcal infections

The majority of gonococcal infections are in low-income and middle-income countries. Gonorrhoea has no symptoms in about 80% of women and 50% of men. Between 2012 and 2016, gonorrhoea notification rates increased by 63% (62 to 101 per 100 000), with an increase in both males (72%) and females (43%).

Penicillin was used to treat STIs until resistance to penicillin developed. Resistance also developed to tetracycline and spectinomycin, and there is increasing resistance to azithromycin. South Africa previously used penicillin to treat STIs. When resistance developed, there was a move to using third-generation cephalosporins (both oral and via intramuscular injection), such as ceftriaxone. Ceftriaxone in combination with azithromycin is currently the recommended treatment for gonorrhoea in most places, but there was decreased susceptibility to ceftriaxone of 1.7 – 4.3% between 2012 and 2016. With increasing resistance to cephalosporins, there is a threat that there will be no drugs available to effectively treat gonococcal infections.

There is a need for new drugs for multidrug-resistant *N. gonorrhoeae*. This could entail repurposing known drugs (e.g. gentamicin, ertapenem and fosfomycin) or developing new drugs with *in vitro* data (e.g. sitafloxacin and delafloxacin). New compounds are ready for investigation (e.g. solithromycin, zoliflodacin and gepotidacin). A clinical trial of zoliflodacin would be starting soon.

The Lancet Infectious Disease Commission in 2017 addressed challenges for research, practice and policy and identified the following major areas for attention: chlamydial infections, emerging drug resistance in gonococcal infections, bacterial vaginosis, STIs in low-income and medium-income countries, and epidemics of STIs in high-income countries in the population of men who have sex with men. Partner management strategies also need urgent attention.

The WHO Global Health Sector Strategy (2016 – 2021) looks specifically at STIs, with a focus on universal health coverage. Services have to be provided to vulnerable populations. A public health approach of integrating services is required so that vulnerable people can be reached and properly managed.

South Africa is entering the second year of its NSP for HIV, TB and STIs (2017 – 2022). Previous strategic plans hardly addressed STIs at all, so the current NSP is a sign of significant progress. The priority areas cover the whole continuum of care: prevention, testing, treatment and care, health systems and surveillance, research and innovation. Laboratory diagnostics need to be improved, because this capability has declined through the focus on syndromic management. High-risk populations need to be identified. There is an urgent need for baseline data so as to set meaningful targets, and to scale up expertise (clinical and laboratory) and resources.

Round-Table Discussion II: One Health

Moderator: Prof Thomas Mettenleiter, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Germany

Panellists:
Prof Thumbi Ndung'u, University of KwaZulu-Natal, South Africa
Dr Alison Lubisi, Agricultural Research Council (ARC), South Africa
Dr Kristina Roesel, International Livestock Research Institute, Kenya
Prof Marietjie Venter, University of Pretoria, South Africa
Prof Rose Hayeshi, North-West University, South Africa

One Health is gaining increasing attention.

In the 1960s, people believed that the world was on the brink of victory over infectious diseases. There were significant advances that eliminated smallpox and rinderpest, which were highlights in the health and veterinary profession. However, the reality is different. Infectious diseases have reached the highest political level as a major concern. The term 'One Health' was taken up in the G20 Summit Declaration of 2017, together with a commitment to strengthen the One Health approach within the G20 while fully respecting the specific mandates of the WHO, OiE and FAO. The political climate is favourable to introducing the topic of One Health to the primary political agenda.

The One Health concept recognises that human health is connected with the health of animals and the environment. This holistic approach is not new but is novel in combatting infectious diseases.

In 2017, directors of G20 public health and veterinary health institutions met for the first time in Berlin to discuss issues related to the One Health concept. For high-income countries, the major One Health issue is AMR. AMR has implications for interactions between human and veterinary medicine, and implications for food and food products, and the environment. AMR is thus clearly a One Health issue. However, for lower and middle-income countries, zoonotic infections are particularly important. More than 60% of existing human infections is zoonotic (i.e. they have an animal source), and the percentage among newly emerging infectious diseases is estimated to be 75%. Five new human diseases appear every year, of which three on average are of animal original. Eighty per cent of agents with potential bioterrorist use are zoonotic pathogens.

The WHO Blueprint of Priority Diseases is a special tool for determining which diseases and pathogens to prioritise for research and development in public health emergency contexts. It seeks to identify those diseases that pose a public health risk because of their epidemic potential and for which there are no, or insufficient, countermeasures. On the 2018 list of diseases to be prioritised under the R&D Blueprint, seven of the eight infectious diseases are zoonotic: Crimean-Congo haemorrhagic fever, Ebola viral disease and Marburg viral disease, Lassa fever, Middle East Respiratory Syndrome (MERS) and severe acute respiratory syndrome (SARS), Nipah and henipaviral diseases, RVF, Zika disease and Disease X. Disease X represents the knowledge that a serious international epidemic could be caused by a pathogen currently unknown to cause human disease, and so the R&D Blueprint explicitly seeks to enable cross-cutting R&D preparedness that is also relevant for an unknown Disease X as far as possible.

The 13 most important zoonoses account for 2.2 million fatalities and 2.4 billion morbidities annually. On average, every third person in the world is infected once a year with a zoonotic agent, which is a huge burden of disease. The most important zoonoses are primarily gastrointestinal infections (mostly parasitic) but include also zoonotic tuberculosis and rabies. The outlook is not expected to improve in the short term. In terms of human and animal interaction, the global demand for meat is predicted to increase drastically within the next few decades, because of rising income levels in increasing numbers of countries. This is particularly relevant for the demand for poultry, which is predicted to more than double in the next 30 years.

The health burden of zoonoses is primarily in low to middle-income countries, and these countries have the least means to address the challenges. High-income countries have a responsibility to help low to middle-income countries combat zoonotic infections.

Zoonotic diseases also have an impact on human nutrition. Food sources can be lost through zoonotic diseases that kill animals used for food. High-income countries can compensate for the loss, but low-income countries cannot. This puts the One Health concept into perspective as extending more widely than the human-animal interface.

We live in a globalised world in which infectious diseases do not respect national diseases. An infection that arises in one place has the potential to spread worldwide within 24 hours. The epidemiology of infectious diseases has thus changed radically in the last few decades due to trade, travel, passive vectors (e.g. vehicles, objects in contact with animals or animal products) and active vectors (e.g. arthropods and birds).

There is a need for more coordinated activity within the G20 and with countries that suffer from the burden of infectious diseases through epidemiology, monitoring, surveillance, research and coordinated best practice.

The G20 Summit Declaration on One Health undertook to support and facilitate the regular exchange of evidence and science-based knowledge in the field of human and animal health, agriculture and the environment. The present conference is one aspect of building what is required to deal with One Health. The last century was characterised by incremental advances, but there is a need to speed up.

Prof Thumbi Ndung'u, University of KwaZulu-Natal, South Africa

Prof Thumbi Ndung'u presented evidence of transmission-virulence evolutionary trade-offs in the spread of HIV-1 subtypes.

HIV viral diversity presents an opportunity to understand how pathogens are transmitted and persist. There are significant differences in replication capacity among subtypes. HIV-1 subtypes show a hierarchy of Gag-protease-driven replication capacity consistent with reported differences

in rates of disease progression and paradoxically with reported differences in transmissibility.

Understanding the genetic characteristics of viruses and other pathogens associated with transmissibility and virulence has important implications for controlling epidemics and predicting outbreaks.

Dr Alison Lubisi, Agricultural Research Council, South Africa

Dr Lubisi gave a presentation on One Health needs for effective RVF control in endemic areas. RVF is an emerging and re-emerging zoonotic disease affecting domestic ruminants (mainly camels) and man. It is caused by RVF virus, a *Phlebovirus* in the Bunyaviridae family. Widespread abortions, hepatitis, death among young animals and teratogenic effects characterise the disease in animals. Humans develop self-limiting flu-like symptoms, which may progress to retinal damage, hepatitis, nervous symptoms and haemorrhagic disease. When RVF affected Somalia in 1997/98, more than half a million people died, and the economy suffered because of its dependence on the export of small livestock.

The transmission cycle of RVF shows that cattle are a big culprit. The disease is also spread by mosquitoes (*Aedes* mosquito). The major cause of RVF in humans is handling aborted material, carcasses, excretions or secretions from infected animals. There are also believed to be wildlife reservoirs of RVF as some wildlife animals have shown symptoms, but these are not as overt as in domestic animals.

The prevention of RVF involves:

- Use of sentinel animals to monitor viral activity in the inter-epidemic period, which is the responsibility of veterinary officials.
- Early-warning satellite imagery involving climatic data collation (i.e. sea-surface temperature fluctuations, resultant high rainfalls and normalised difference vegetation index measurement), which is the role of climatologists.
- Vector control by conservationists and environmentalists.
- Vaccination by veterinary and medical officials (live attenuated, inactivated or clone 13); however, there are still no vaccines against RVF for humans.

The control of RVF during an outbreak entails:

- Safe carcass disposal by veterinary officials and environmentalists.
- Quarantine and movement restrictions, which is the function of law enforcement.
- Ban on livestock slaughtering, enforced by veterinary officials.
- Ring vaccination by veterinary officials.
- Education by veterinary officials and social groups.

The gaps with respect to knowledge and control of RVF include:

- Virus maintenance hosts during the inter-epidemic periods. In East Africa it is believed that there must be other inter-epidemic hosts other than mosquito eggs that act as reservoirs, leading to outbreaks occurring when the environmental conditions are conducive.
- Drivers of emergence and re-emergence in different geographic regions. In Southern and East Africa, it is known that RVF outbreaks occur after high rainfalls, but the epidemiology in West Africa is not well understood.
- Laboratory diagnostic tests are needed. There are still no rapid tests available; the tests lack markers to differentiate infected from vaccinated animals; and there is a need for tests with application in all species. Only one company has commercialised RVF serological tests, but the tests need to be more sensitive.
- Effective vaccines for application in all susceptible animals and man.
- Vaccine banks for emergency responses.

One Health needs in relation to RVF include:

- Joint projects for simultaneous execution spatio-temporally for meaningful epidemiological studies.
- Resource sharing (e.g. transportation and manpower).
- Development and use of multi-species tests.
- Development and use a single vaccine in all susceptible species, including man (e.g. MP12 is promising and clinical trials are ongoing).
- Logistical and coordinated execution of control measures during outbreaks, with clear responsibilities and commitment.
- Sharing of samples for research purposes and laboratory test validation exercises;
- Regular inter-laboratory test comparisons (ISO17025 compliance).
- Skills transfer, including research methods, insect identification and clinical sign recognition.

- Regular information sharing, including early warning information and new epidemiological information.

The One Health approach could address the challenges with RVF.

Dr Kristina Roesel, International Livestock Research Institute, Kenya

The International Livestock Research Institute in Kenya is part of a consortium of 15 agricultural research institutes that came together in the 1970s to conduct agricultural research to improve global food and nutrition security and to reduce poverty in low and middle-income countries. Most of the centres focused on crop-based foods. The International Livestock Research Institute is the only one that looks at livestock and is thus by default a One Health institute through securing human access to food and income by improving animal health. In the first 30 years of the existence of the institute, the focus was on haemoparasites (East Coast fever and trypanosomiasis, which are cattle diseases). Developing vaccines and control strategies can be a lifelong activity; in the past 15 years the institute has therefore broadened its approach to include work on food safety, zoonoses and emerging infectious diseases. For the past two years, the institute has had an Animal and Human Health Programme that follows a multidisciplinary and multisectoral approach.

For instance, the approach to food safety has been to look at the livestock value chains, from production to consumption, together with animal nutritionists, human nutritionists, economists and sociologists, using risk-based methods developed in high-income countries, and adapting social science methods to close data gaps. Jointly with scientists working on climate change and data managers, the International Livestock Research Institute works on RVF, for which climate-based data are important in predicting outbreaks. Models have been developed to forecast RVF outbreaks in Kenya.

Moreover, the International Livestock Research Institute is working with national and international institutions on emerging infectious diseases in pigs, which are of growing importance in Africa. The institute is also involved in abattoir-based surveillance. The social sciences are important in assessing the human health burden, animal health burden and economic burden of disease.

Prof Marietjie Venter, University of Pretoria, South Africa

Most zoonotic diseases circulate in the environment during inter-epidemic stages. Unless the diseases are addressed at that stage, they can cause major outbreaks among animals, which spill over into the human population. There are very short opportunities to act to combat the diseases. If outbreaks can be predicted in the environment before humans are infected, it is possible to take steps against the disease, and there is more time to respond (e.g. by vaccinating animals or controlling mosquitoes).

One of the problems with zoonotic diseases is that many of them present symptoms similar to common diseases. South Africa has experienced many epidemics of zoonotic diseases, including brucellosis, Crimean-Congo haemorrhagic fever, Q-fever, West Nile fever, rabies and avian influenza, but these are often missed because they present like common diseases. It takes a specialist to be aware of and diagnose these zoonoses, as well as specialised surveillance to fight the diseases.

The approach at the University of Pretoria is to do surveillance in sentinel animals and humans. In animals this surveillance looks for neurological signs in horses, livestock and wildlife; bird fatalities; abortion and death in young animals; and signs or viral haemorrhagic fever (VHF). Human syndromic surveillance includes febrile disease, neurological signs, arthralgia and VHF. If signs are found among humans, there is a search for vectors in the area where the cases occurred. This approach has been very successful in describing West Nile virus (WNV).

South Africa has several One Health initiatives to try to bring together human and animal aspects. The National One Health Forum was launched in 2014 involving the Department of Agriculture, Forestry and Fisheries, the DoH, the ARC Onderstepoort Veterinary Institute, the National Institute for Communicable Diseases, state veterinarians from across the country, medical schools, academia and some private veterinarians and doctors. The forum meets quarterly, and outbreak response teams meet monthly with representatives of the various sectors. There was an exercise in 2016 to prioritise some of the zoonotic diseases, which proved complicated, as it is difficult to decide which diseases are more important than others; however, it is important to decide which diseases the government should focus on and invest in. The Annual One Health Conference was launched in 2016 to coincide with celebrating International One Health Day. In November

2017, the forum participated in a WHO Joint External Evaluation as required by the International Health Regulations. In the evaluation, South Africa was commended for having many things in place for zoonotic disease preparedness and response. The areas identified for action were training and awareness for doctors in early detection of zoonoses in humans, and an approved official policy for One Health.

Prof Rose Hayeshi, North-West University, South Africa

The DST/North-West University (NWU) Preclinical Drug Development Platform is a state-of-the art national facility for the innovation of pharmaceutical development focusing on preclinical drug development, formulation and diagnostics. This is a joint initiative between the DST and NWU.

Preclinical drug development was identified as a strategic development area for NWU. The platform is based on the implementation of expertise; it is at heart an initiative that is intended to teach postgraduates and to supply expertise and innovation to scientists and businesses, both locally and internationally. This includes education in the One Health concept.

The development of a vibrant pharmaceutical industry in South Africa forms part of the DST's Bio-economy Strategy. Preclinical studies are a crucial step in the development and registration of any therapeutic product. Pharmaceutical companies want to avoid late-stage failures or drugs labelled for restricted use following approval. Thorough preclinical studies can contribute to early decisions about further development, thus saving time and money and increasing the success rate of the project.

Discussion

Question (Prof Thomas Mettenleiter): Is there a gap in education with respect to One Health in order to make the concept a household name? Does this require more emphasis on education at graduate, postgraduate and continuing education levels?

Response (Dr Alison Lubisi): We are doing well in veterinary public health. Veterinary students understand that One Health is where human and animal health meet. One Health features well in the veterinary curriculum in South Africa, but it seems that more effort may be required to emphasise the importance of One Health in training in other disciplines.

Response (Prof Marietjie Venter): The concept of One Health is often presented at undergraduate level and then forgotten. In the medical context, there is a gap in that doctors do not always appreciate the role of One Health. Even after a decade of surveillance and explaining to the DoH why it is important to look at neurological cases, there is still a lack of understanding. There is a need to raise awareness, and for medical and veterinary scientists and practitioners to meet and talk more. From the perspective of animal science, there is an effective surveillance system for rabies, for example, but there are still human deaths from rabies, which represents a failure of public health and points to lack of communication between doctors and veterinarians. One Health receives sufficient attention at undergraduate level, but at postgraduate level it is important to keep reminding professionals in different associated disciplines to communicate with one another.

Response (Dr Kristina Roesel): Undergraduate and postgraduate students, as well as scientists need to be more open to communication, collaboration and working with scientists in other fields towards the common vision and goal of One Health. Specialists sometimes lose touch with the overall vision for their work and what other disciplines could contribute.

Response (Prof Thumbi Ndung'u): The One Health issues are well covered in veterinary curricula, but there may be a gap in the approach of human medicine colleagues, where there seems to be a lack of emphasis on cross-disciplinary One Health issues. The animal and human health fields need to work together better.

Question (Prof Thomas Mettenleiter): Medical doctors tend to define One Health in terms of human health. This attitude needs to change. Veterinarians tend to be the leaders in One Health because they are used to working with multiple species. However, the environmental aspects of One Heath are generally completely overlooked. How can this gap be closed?

Response (Prof Rose Hayeshi): One of the gaps in the curricula for life and natural scientists is that the translation aspects of fundamental research are not discussed, which may be why there is so little awareness of One Health. Collaboration with scientists in different areas should feature more prominently in research strategies.

Question (Prof Thomas Mettenleiter): How embedded is the One Health concept in veterinary, medical and natural science curricula?

Response (Prof Marietjie Venter): Students in medical and veterinary science at her university learn the One Health concept. It would definitely help to introduce the concept to curricula in the natural sciences, such as microbiology, zoology and biochemistry. Basic scientists need to see the wider implications of their field for addressing human, animal and environmental issues.

Response (Dr Alison Lubisi): Veterinarians are aware of the implications of animal disease for human health, but there is less focus on environmental aspects, which ought to become ingrained (e.g. discarding of drugs). Perhaps there is a need for a tailor-made One Health subject in university curricula that encompasses the broad scope of the concept and shows how issues are interlinked.

Response (Prof Marietjie Venter): The aim of the International One Health Day is to encourage students to participate in events or compete in competitions in teams that include veterinary, human science and environmental students or practitioners. The initiative is being advertised on social media. Since being launched in 2017, there have been 17 events across Africa, 20 in Europe and 20 in the USA. This is a way of involving students and encouraging participation online.

Question (Prof Thomas Mettenleiter): Hardly any of the audience members have heard of the International One Health Day, which falls on 3 November. Academics should bring this to the attention of their students and colleagues.

Question (Dr Shevin Jacobs): There is a need for infection prevention and control initiatives at both pre-service and in-service levels. In human health pre-service training, there is considerable emphasis on infection prevention and control, although this does not happen quite so well in practice, as seen in outbreaks in West Africa, for example, of human-to-human infections. In the case of zoonotic diseases, transmission occurs from animals to veterinarians, or from veterinarians to other human subjects. From the human health perspective, WHO has a number of initiatives to

prevent human-to-human transmission, for example 5 May is World Hand Hygiene Day. Uganda has strong governmental support for a One Health platform, but it is not certain that what is learnt in pre-service institutions is practised by veterinarians in the field. Are there One Health tools for colleagues in animal health that can be used for in-service training to ensure best practice in infection prevention and control and to minimise the transmission of infection from animals to humans?

Response (Dr Alison Lubisi): The only way to address the human, animal and environmental aspects of One Health is to establish clear and binding policies. Individual egos often hinder research and hamper progress. It is heartening that One Health forums are in place. There is now a need for legislation, regulations and protocols (e.g. how to sample bats without driving them to extinction). Bureaucracy also often inhibits research.

Response (Prof Marietjie Venter): Politicians and government officials need to take more responsibility for promoting and institutionalising the One Health approach. Veterinarians are at greatest risk of zoonotic infections. There are training materials specifically from Uganda developed by the Field Epidemiology and Laboratory Training Programme, and there is willingness to implement this in other African countries as well. The US Defense Threat Reduction Agency is keen to do One Health training. Ultimately One Health is the responsibility of national governments in order to protect their populations. When veterinarians go into the field, they often forget that they can be infected by zoonoses. Basic training to use gloves and masks when handling animal samples is very important. This is perhaps the responsibility of state veterinarians.

Question (Prof Sabiha Essack): One Health is cross-cutting and requires coordinated implementation from the various sectors that are responsible. One Health initiatives tend to be reactionary. Very few countries have a One Health policy or overarching national health plan. Is there merit in an assistance-based approach, ranging from policy and legislation to practice on the ground? If so, how would this be implemented? Another factor is that without enforcement capability or accountability measures, One Health legislation would not be effectively implemented.

Response (Prof Thumbi Ndung'u): The issues are difficult. The first thing would be to create awareness of One Health and the need for interdependence between disciplines. Such an approach would lead to people from

different disciplines interacting more, and to communication between the veterinary, medical and environmental fields. It should be considered work in progress for disciplines to work together. When there is an outbreak, the various disciplines are forced of necessity to work together. We are making progress in the right direction, but we still have a long way to go.

Response (Prof Rose Hayeshi): The One Health concept is inherently interdisciplinary. Teams that work across the One Health continuum already exist and need to be strengthened.

Response (Dr Kristina Roesel): Apart from legislation and policy, there is a need for creating an enabling environment for One Heath, for instance, to facilitate implementation and enforcement. There are already regulations for food safety, which is a One Health topic, but these are not always enforced because of issues such as high-level corruption, and lack of human capacity and laboratories.

Response (Prof Marietjie Venter): There are many policies in place, especially in South Africa, but education is also required starting at school level. South Africa has school education initiatives associated with International One Health Day. There are also initiatives to educate individuals. In KwaZulu-Natal, for example, rabies has been a big issue, but individuals drove the rabies campaign and have made a big difference in trying to eradicate rabies in animals. Similar actions are required in other parts of the country. One Health is a governmental responsibility, but individuals also have a role to play. Universities and institutions should find champions for One Health.

Question (Prof Quarraisha Abdool Karim): In addition to awareness raising, there is a need for training for preparedness for medical or zoonotic outbreaks, rather than preparing for disaster management. There is a need to bridge the gap of training for medical interns who are appointed in areas other than where they were trained, with a different disease profile. Once they become familiar with the diseases that occur in the area, they are better able to deal with them. This needs to be done annually with each new group of interns to ensure that they are fully prepared to deal with disease outbreaks. Some interns, for example, do not know how to take samples for rabies. With closer networking, the One Health concept can be achieved. Veterinarians have skills that medical practitioners could use. There is a need to assist one another.

Summary of issues raised (Prof Thomas Mettenleiter):

- Thanks to the panel members for their participation and serving as multipliers to raise awareness of the One Health concept. It is a recommendation to the academies to embrace the One Health concept and act as multipliers themselves.
- One Health issues need to be featured more prominently in education at university and school levels. Current education initiatives need to be expanded and include practical advice.
- One Health is not just a combination of medicine and veterinary science, but also involves environmental and societal issues.

SESSION 5: BIG FOUR: HIV, TB, MALARIA, HCV

Facilitator: Prof Koleka Mlisana, University of KwaZulu-Natal, South Africa

Malaria and West Nile Virus Co-Infection Amongst Febrile Patients Attending a Tertiary Hospital in Abuja, Nigeria (Mr Aina Kehinde Oluwasegun, University of Ilorin, Nigeria)

Arboviral diseases have risen considerably, increasing the risk of co-infection with other endemic diseases. Epidemiological data suggest a greater incidence of negative effects on pathogen-specific host immune responses during co-infection. Among the prevalent infectious diseases in the world, mosquito-borne parasites and viruses are frequently co-endemic in subtropical areas. Malaria transmission still persists in 95 countries, accounting for over 214 million new cases worldwide in 2015 alone.

Mosquitoes are carriers of various pathogens that cause disease in human, including Dengue, Chikungunya and West Nile fever. An estimated two billion people live in areas where these diseases persist. Co-infection among these diseases is possible in geographical locations where the respective vectors co-exist.

Arboviral infections and malaria are acute vector-borne diseases, representing the most common arthropod-borne diseases with febrile symptom in humans. Concurrent infections are widely under-reported in sub-Saharan Africa. Arboviral infections are often misdiagnosed with malaria due to their similar clinical presentation.

WNV fever is a widespread mosquito-borne zoonotic arbovirus of global importance, and concurrent infections are a factor in regions where their endemic areas overlap extensively. WNV is a *Flavivirus* single-stranded RNA virus with two genetic lineages. Lineage 1 has three clades (1a, 1b, 1c) and infects humans, birds, mosquitoes, horses and other mammals.

WNV was first isolated in the West Nile district of Uganda. In 1950 the ecology of the disease was studied in Egypt. Additional outbreaks occurred in 1951 – 54 and 1957 in Israel; in 1962 and 2000 in France; in 1973 – 74 in South Africa; in 1996 in Romania; and in 1998 in Italy. WNV represents one of the commonest agents of febrile illness. In its most common forms,

WNV resembles influenza. Most infections resolve within two to six days, but persistent fatigue can occur.

Malaria is currently endemic in more than 100 countries worldwide and the leading cause of illness in Africa and tropical countries. The global burden of disease is formidable. WNV, malaria and febrile illness have an overlapping geographic distribution in sub-Saharan Africa. There is limited knowledge of the effect of co-infection on the host immune response. WNV and malaria are usually implicated as aetiologies of acute febrile illness in some African countries apart from Nigeria. There was an inconsistent sero-survey of WNV/malaria co-infection in Nigeria.

The presentation described a study of WNV/malaria co-infections in patients with acute febrile illness attending the University of Abuja Teaching Hospital, its prevalence and associated risk factors. The following research questions were set for the study:

- What is the prevalence of WNV/malaria infections and associated risk factors among patients with acute febrile illness?
- What is the clinical and epidemiological significance of diagnosing WNV in a setting endemic with malaria?

The study was a hospital-based cross-sectional study. Purposive random sampling was used to assemble a sample of 162 participants. The inclusion criteria were those who present with febrile illnesses regardless of onset and duration of illness, and consent to participate voluntarily in the study. The exclusion criteria included apparently-healthy individuals without any trace of ongoing febrile illness in the last three weeks, as well as critically ill individuals.

For each participant, 3 mL of venous blood was collected in tubes without anticoagulant and centrifuged at 3000 rpm for 15 minutes to obtain the serum. The sera were separated and used for the IgM Elisa assay.

The results generated from analysis and data obtained from the questionnaire were analysed. The chi-square test was used to determine the level of significance in the occurrence of malaria parasitemia and WNV IgM in association with categorised variables at a CI of 95%. P-values were reported to be statistically significant at <0.05.

Of the 178 samples studied, 113 (prevalence of 66.1%) were seropositive for WNV virus IgM. Of these 113, 38 (33.6%) were co-infected with WNV and malaria parasites. The prevalence of malaria in this study was 28.07%. All malaria parasites were *P. falciparum*.

Based on demographic factors, 62 of the 113 positive cases of WNV IgM were female participants with a prevalence of 62/113 (54.9%), which was higher than that of their male counterparts. Seropositivity was observed to increase from participants who were within the age range of 25 – 36 years (36%). Regarding occupation, IgM to WNV was detected in the highest prevalence (20/23) among farmers, while the lowest prevalence (16/47) was recorded among the unemployed participants.

The concerted efforts by WHO to combat malaria have resulted in its mortality falling by 42% globally. However, the issue of the involvement of co-infection with one or more arboviruses has called for taking febrile conditions more seriously than before.

WNV currently poses a significant threat to human populations in Africa as well as the entire globe, and vector habitats appear to be increasing; therefore, new epidemics of WNV could be looming. The prevalence of malaria and WNV co-infection in this study is an indication of poor vector control measures against mosquito vectors in the community. There is a high sero prevalence of WNV in the study area being sampled, which might be indicative of an ongoing infection. The seasonality in the activity of the mosquito vector (*Culex* spps) has a large influence on the activity of WNV.

This study recommended that:

- In the absence of a vaccine, the only way to reduce infection is by raising awareness of the risk factors and educating people about the measures they can take to reduce exposure to the virus.
- Clinicians and public health physicians should be sensitised on the proper management, diagnosis of WNV infection and the role they play in acute febrile illnesses.
- Public health educational messages should focus on reducing the risk of mosquito transmission.
- Integrated mosquito surveillance and control programmes should be carried out in this area.

- Further studies should identify the local mosquito species that play a role in WNV transmission, including those that might serve as a bridge from birds to human beings.

Discussion

Question (Ms Alison Lubisi): In terms of surveillance, do you involve people who look at birds?

Response (Mr Aina Kehinde Oluwasegun): No, the study only involved febrile patients. There is no intention to involve people working on birds.

Question: What is the next step in your research as a young researcher?

Response (Mr Aina Kehinde Oluwasegun): The research suffers from a lack of diagnostic facilities to confirm the presence of virus in samples. It is hoped that collaboration and funding can be found for this research.

Lipids Go Viral: Deciphering the Function of Lipids and Lipid Droplets in HCV Infection (Dr Eva Herker, Leibniz Institute for Experimental Virology, Germany)

The global hepatitis C virus (HCV) rate has reached epidemic proportions with an estimated 150 million people infected, which represents 3% of the world population. Infection rates vary from below 1% to over 10% in different parts of the world. Better treatment has become available for HCV, but worldwide only 5% of people are aware that they are infected, and of those only 5% are treated, and most are treated with old medicines, since the new antivirals are generally too costly for most people to afford. Even when people are treated, there is still the risk of disease progression or reinfection.

HCV replication is closely tied to the host cell lipid metabolism. Lipid droplets have emerged as key organelles for HCV replication, and it has been proposed that they serve as virion assembly platforms. The viral capsid protein core localises to lipid droplets, recruits viral RNA replication complexes, and initiates the assembly of progeny virions at lipid droplet-associated membranes of the endoplasmic reticulum. It has previously been shown that a host protein involved in lipid droplet biogenesis serves as a key regulator of viral replication. The fact that HCV selectively targets

a subset of lipid droplets points to hitherto unrecognised specificity. However, the virus targets lipid droplets and the mechanistic details of the late stages of HCV replication are still ill defined. Proteomic and lipidomic approaches are used to elucidate in molecular detail the role of lipids and lipid droplets in HCV replication.

Discussion

Question (Prof Thomas Mettenleiter): Did you test any of the other Flaviviridae viruses and find similar results?

Response (Dr Eva Herker): The vasicular structures induced by other Flaviviridae viruses look different from HVC. It seems that specific lipids are required. This will be investigated.

Question (Prof Thomas Mettenleiter): Will you look at inhibitors?

Response (Dr Eva Herker): We would try, but unfortunately we do not have inhibitors for the fatty acid elongases.

Question (Prof Anna Kramvis): Would there be competition between HVC and HVB?

Response (Prof Eva Herker): There would perhaps be competition over host cell resources; the fittest virus would use the resources.

Prevalence of Human Papilloma Virus and Factors Associated with oral and Oropharyngeal Squamous Cell Carcinoma Among HIV-1 Infected Patients Attending Mulango Hospital, Uganda (Dr Annah Margret Biira, Makerere University, Uganda)

Human papilloma viruses (HPVs) are a heterogeneous group of small non-enveloped epitheliotropic DNA viruses. HIV-1 infected individuals have two to three times higher chance of prevalent oral HPV infection compared to non-HIV counterparts.

Dr Biira's research determined the prevalence of HPV 16, 18 and factors associated with oral (OSCC) and oropharyngeal squamous cell carcinoma (OPSCC) among HIV-1 infected patients attending Mulago Hospital.

The study confirmed the role of HPV 16 and 18 in OSCC and OPSCC pathogenesis in the Ugandan population. The results suggest that HPV is an aetiological factor responsible for the high occurrence of oral and oral pharyngeal squamous carcinoma in HIV infected people.

A bigger study is underway, where all HPV genotypes will be assessed and other sociodemographic factors considered using a higher statistical power.

Facilitator: Prof Stefan Kaufmann, Director, Max Plank Institute for Infection Biology, Germany

The Development of a Nanomedicine-Based Drug Delivery System with the Potential to Improve Tuberculosis Therapy (Dr Madichaba Chelopo-Mgobozi, North-West University, South Africa)

TB treatment challenge

TB is still a major health threat that burdens a large number of poor communities in the developing world and is one of the major causes of death amongst a group of infectious diseases even though there are effective drugs approved for its treatment.

Poverty-related diseases are the major cause and consequence of considerable poverty in developing countries, particularly those in sub-Saharan Africa. South Africa is a TB high-burden country. Fighting these diseases remains one of the most effective ways to alleviate poverty and promote economic progress in these countries.

Current treatment comprises a fixed dose combination of the following four potent drugs: RIF, isoniazid (INH), pyrazinamide and ethambutol, approved by WHO, to be taken daily for a period of up to six months.

The failure to control or reduce the number of TB cases is aggravated by the high dosage, long treatment duration, development of side effects and poor patient compliance, typically leading to the development of drug-resistant TB strains that present yet more challenges in the treatment of TB.

The use of drug delivery systems

Along with all the strategies used to reduce the burden of TB, it should be mentioned that the existing anti-TB drugs are still effective.

Overcoming technological drawbacks of these therapeutic agents and improving the effectiveness of the drug by targeting the infection reservoirs remain the central aims of pharmaceutical technology. The formulation of potent anti-TB drug within suitable drug delivery systems (DDS) appears to be one of the most promising approaches for the development of a more effective treatment, with which patients can comply. A DDS is a formulation that assists the transport of a drug in the living body and enhances its efficacy and safety through the control of the time and location of its release. Such systems generally overcome the disadvantages of free drugs and enhance their activity.

Developing and designing a suitable DDS is a key area of extensive research and takes into consideration many factors, such as the target tissue and the type of drug being transported. The question is whether it can reach the target tissue and protect the drug from undesirable degradation in the body.

The goal in the use of drug delivery formulations is modulation of the pharmacological profiles, namely the pharmacokinetics and pharmacodynamics of the therapeutic agents to ensure clinical potential. Therefore, to design an efficient delivery system, important principles such as drug stability, drug solubility, drug safety, biocompatibility, therapeutic efficacy and industrial scale-up need to be taken into consideration.

The field of DDS dates back to the 1950s. After the emergence of nanotechnology in the 1970s, it became the main driver in the growth of DDS and led to the availability of various delivery platforms that exist in the nanoscale. Nano-size particles possess distinct chemical and physical properties that offer a number of unique advantages over micrometre-sized particles, particularly in drug delivery. Nano-size particles also have very high surface area per unit volume, and this has had a significant influence in the field of drug delivery, where most drugs with generally poor bioavailability attained significantly improved bioavailability when encapsulated in nanoparticles (NPs). This attribute of cellular uptake is essential for the treatment of intracellular pathogens, such as TB infection.

Nano-based DDS have brought transformation to the field of pharmaco-therapy through their capability to modify the pharmacokinetics properties of conventional drugs, which includes the extension of the drug circulation time, increased half-life of the drugs and reduced toxicity.

Several DDS have been explored for TB therapy and had the following results in rodents:
Polylactic-co-glycolic acid (PLGA) NPs with anti-TB drugs: This system improved the bioavailability, controlled the release of drugs, reduced the drug dosage, and was easily taken up by alveolar macrophages.

- Liposomes with anti-TB drugs: This system showed overall increases in anti-TB activity with a significant decrease in toxicity, but cannot be administered orally;
- Pheroid® vesicles with anti-TB drugs: This system exists in both the micro and nano ranges. The systems improved intestinal absorption of drugs, increased their half-life, enhanced the bioavailability of drugs, and can be administered orally.

However, uncertainty over safety and toxicity, high costs and challenges of scaling up production made pharmaceutical industries reluctant to develop them for poverty-stricken countries. These drawbacks apply to many other DDS. There has been a lack of human trial studies to evaluate the effect of polymeric NPs on anti-TB drugs.

Despite the effectiveness of polymeric NPs and lipid-based DDS for anti-TB drugs, sufficient data from clinical trials is still required in order to pave the way to bring them into the market. It was predicted that the use of nanomedicine to deliver effective conventional therapeutic agents would facilitate a faster transition of effective DDS-formulated therapy to clinics for better control of poverty-related infectious diseases. However, the advancement of these DDS for TB is limited by pitfalls such as bioaccumulation, cumulative toxicity and side effects associated with these nanomedicines. The lack of extensive research studies on safety and long-term stability hinders the progress of new medicine formulations to human trials. Other major hurdles in advancing these delivery technologies, especially for the improvement of TB therapy to the clinical stage, include the high cost of the drug-delivery materials, the inability to conduct large-scale production and the removal of residual organic solvents.

The high cost of developing effective drug-loaded DDS for poverty-related infectious diseases leads to reluctance by pharmaceutical industries to advance them to the market. Although new potential DDS continue to be proposed for the improvement of PK for current anti-TB drugs, novel strategies such as the combination of two effective DDS do not seem to have been explored yet.

Hybrid DDS

Hybrid delivery systems combine the advantages of polymeric NPs and lipid-based systems and have emerged as a robust and promising delivery platform, resulting in potentially robust drug delivery technology, with excellent stability, sustained drug release and enhanced drug delivery.

Dr Chelopo-Mgobozi developed a hybrid DDS comprising three major components, namely the drug, PLGA and a lipid bilayer shell. A hybrid system makes the most of the unique attributes of each technology, with the advantages of improved stability, enhanced compatibility and more superior *in vitro* cell efficacy.

The recent approach in dealing with the TB problem has therefore been to focus on DDS that have previously been investigated (independently) to improve the current TB therapy to reduce dosing frequency and shorten the treatment period.

The combined PLGA NP-Pheriod® hybrid DDS was hypothesised to create a powerful novel system to more effectively improve PK than individual systems, in which the drug would be absorbed faster and then be slowly released in a living organism:

- Polymeric NPs are composed of biodegradable PLGA polymers and demonstrate outstanding structural stability and controlled drug-release mechanism;
- Pheroid® technology is a lipid-based method of delivering drugs. It is a submicron emulsion consisting of essential fatty acids and has exceptional ability to enhance the absorption of numerous drugs.

This work was a collaborative initiative between the NWU and the Council for Scientific and Industrial Research (CSIR).

Preparation methods and characterisation

The preparation was based on making an emulsion, and solid NPs were produced. Double emulsion followed using a spray-drying technique to produce solid NPs. The polymer is usually dissolved in the first or internal emulsion; depending on the solubility of the drug, which could be either in the O or W phase. The addition of excipients ensures better surface properties of the drug such as its shape and surface charge. The final double emulsion is subjected to a spray-drying technique. To produce the Pheroid® vesicles, single nitrous oxide saturated water with FA oil was homogenised to form an emulsion.

Various combination methods were explored

- In the post-mix, preformed Pheroid® vesicles were combined with the preformed NPs through vortex to obtain the hybrid system.
- The pre-mix method involved adding preformed NPs to at least one constituent of the Pheroid® vesicle prior to their production.

Characterisation showed that the pre-mix method resulted in a more stable hybrid system with higher zeta potential (ZP) than using the post-mix method. In the pre-mix method, electrostatic interaction was responsible for the successful combination of NPs and Pheroid®. In addition to measuring size and ZP, visual analysis was done through transmission electron microscopy (TEM) and confocal microscope.

Pharmacokinetic studies and results

In PK studies, healthy BALB/c mice were used and were divided into three per drug, administered via oral gavage. The first group received the free drug; the second group received the drug formulated in a NP; and the third group received the drug formulated in the hybrid DDS. The aim of the hybrid system was to entrap the anti-TB drug (INH or RIF) within hybrid DDS and evaluate its PK parameters *in vivo*. The mice were euthanised at ten different time points. Plasma and various organs or tissue were collected and analysed with LC tandem mass spectrometry to establish the drug levels.

The following PK results were obtained:

- There was found to be no statistically significant difference between the PK parameters – C_{max} (maximum concentration of the drug achieved in the plasma following dose administration); AUC (area under the plasma drug concentration-time curve); T_{max} (time at which C_{max}, is attained) and $t_{1/2}$ (half-life) for both INH and RIF formulations. However, the probability of superior PS values showed that the hybrid (NP-Pheroid®) formulation had an effect on $t_{1/2}$, AUC and C_{max} compared to free RIF and NPs by medium practical significance.
- There were no statistically significant variations caused by the RIF hybrid system on either AUC and C_{max}. However, the PS values indicated that the RIF hybrid system had a large effect sizes (PS > 0.71) on the AUC, C_{max} and $t_{1/2}$ in the liver, intestines and kidneys.
- The RIF hybrid formulation significantly increased $t_{1/2}$ of free RIF from four to 16 hours in the kidneys (P = 0.042). The effect size of RIF hybrid formulation on $t_{1/2}$ is also large in comparison to both RIF NPs and free RIF.

Another study was conducted to measure the effect sizes, which provided a different perspective on the results, showing a medium effect in the plasma on the PK parameters and the potential positive outcome of hybrid systems.

Conclusion

The design of effective DDS is a key to ensuring improved activity of current TB treatment and reducing the risk of developing resistant strains.

The fabrication of the hybrid PLGA NP-Pheroid® DDS through the pre-mix method proved to be more stable through electrostatic interaction. Co-localisation was proved by sizing technique, TEM and confocal laser scanning microscopy (CLSM).

NP-Pheroid® did not show any significant statistical difference on the PK parameters of the INH and RIF drugs in the plasma. However, this novel hybrid DDS reduced RIF T_{max} and increased the retention of RIF in the lungs.

The estimated probabilities that the hybrid system improved certain of the PK parameters in comparison to the free drug and drug in NPs served as effect sizes to establish the practical or clinical relevance that the system could have.

Discussion

Question (Dr Alison Lubisi): Some drugs have specific prescriptions for administration (e.g. to be taken after a meal) to optimise their efficacy. Could a DDS circumvent such prescriptions and ensure that the drug is properly absorbed whenever it is taken.

Response (Dr Madichaba Chelopo-Mgobozi): This has not been investigated but should be. It might be possible to design a DDS to address these challenges.

Question (Genevieve Mezoh): There have been many papers about DDS. Are any such systems being used?

Response (Dr Madichaba Chelopo-Mgobozi): DDS are being used for cancer but not for infectious diseases. There are some DDS in clinical trials.

Question (Dr Norbert Heinrich): It was mentioned that the novel hybrid increases the retention of RIF in the lungs. Is there any idea of where, or in which compartment cell in the lungs the RIF is retained?

Response (Dr Madichaba Chelopo-Mgobozi): The study only looked at the tissue of the lung as a whole, not at the exact compartment. This would require a further study. It would go into the macrophages.

Question (Dr Norbert Heinrich): RIF is highly bioavailable. Have you considered working with drugs that have problems with bioavailability (e.g. injectable drugs are not orally bioavailable; and some of the drugs in development that need formulation to make them bioavailable).

Response (Dr Madichaba Chelopo-Mgobozi): The study investigated only two drugs (INH and RIF), which is not representative of all the drugs available to treat TB. Investigating DDS for other drugs would be a possibility for the future.

Importance of Human Proteins for Survival of Human Malaria Parasite *P. falciparum*: An Unexpected Achilles Heel (Dr Jude Przyborski, Heidelberg University Hospital, Germany)

Dr Przyborski presented some of his unpublished work, about which he was very excited.

The presentation would look at the way in which the parasite communicates with the host cell, and how this potential weakness could be used to attack a parasite.

When a parasite invades and proliferates in human erythrocytes, knob structures form on the surface of the infected erythrocyte. The parasite changes the biochemical and physiological properties of the host cell in order to live within the host cell, which is usually enucleated, does not carry out any protein synthesis, and is poor in nutrients. Another modification of the host cell is the formation of knob structures on its surface, within which there are a number of parasite-encoded proteins, the most important of which is *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1). These proteins stick to various receptors on the endothelial cells in various tissues of the body and within blood vessels, leading to blockage of the blood vessels with infected erythrocytes. Non-infected erythrocytes are no longer able to continue to perform their function of transporting oxygen. Patients become hypoxic and ultimately die, especially if this occurs in the brain. Protein transport is responsible for the pathology of malaria when the parasite transports proteins to the surface of the cell.

The parasite exports over 400 proteins to the host cell, but the function of a large number of these proteins is unknown. Dr Przyborski's laboratory studied host cell modifications; how proteins travel to their destination; the signals that direct them in that direction; what molecules are involved in the process; and what functions they carry out once they get there. Parasites have to devise new mechanisms to deal with new situations, all of which are a potential Achilles heel that researchers could target to combat the disease.

The parasite places itself in a difficult situation when it invades an erythrocyte. If the basic secretory system of a large number of eukaryotic cells is studied, it is found that proteins insert ER carried in vesicles to the Golgi, where they are spat out or inserted into the plasma layer.

When the parasite invades blood cells, it surrounds itself with a third membrane, the parasitophorous vacuolar membrane. Any parasite protein that wishes to move to the surface of the cell has to cross the membrane. Additionally, once the parasite protein reaches the erythrocyte, it cannot carry out any protein transport itself, so the parasite has to export a secretory system into the host erythrocyte to carry out this function, or the parasite needs to use the proteins that are already there.

The first research question that Dr Przyborski started to address several years ago was how parasite proteins get across the parasitophorous vacuolar membrane. It is now known that a parasite-encoded translocon sits within the membrane and forms a conduit through which exported proteins can travel. The exact mechanism is not certain, but it seems to be important. It was also known from experiments that Dr Przyborski did at Marburg that to get through the translocon, called PTEX, parasite proteins need to be unfolded, which is common for many protein translocons.

The question thus arises what will happen to the unfolded protein when it reaches the other side of the membrane, since proteins need to be folded correctly in order to carry out their biological function. The proteins have to be refolded, and probably need some other factor that blows them through the translocon.

The factors required on the trans side of the parasitophorous vacuolar membrane were then investigated. In order to study this, the existing GDP assay was improved (GDP is an exported protein that is used as a surrogate for protein export). Tetanolysin was used, which inserts itself into the erythrocyte as a membrane and lyses it so that all the erythrocyte contents can be sucked out. The erythrocyte contents are washed to get rid of the residual protein. This allows the composition of the cell to be changed through the media placed on top. The composition of the trans side of the parasitophorous vacuolar membrane can thus be changed. By doing so, it was hoped that factors on the trans side of the membrane could be identified that were required for protein translocation.

The first experiment was to use erythrocyte cytosol derived from non-infected erythrocytes. This was incubated and then separated into the pellet fraction (the non-exported fraction) and the exported fraction. It was found that there is a factor in non-infected erythrocyte cytosol that is

required for protein translocation. This would have to be a human factor and is likely to be a protein.

The next experiment was to trypsinize erythrocyte cytosol to kill the activity of proteins, and then to put it back on to the cell system. It was found that the translocation efficacy was lost from the fraction. It could thus be concluded that there is a human protein within erythrocyte cytosol that is required for protein translocation. Further experiments showed that the protein requires hydrolysed ATP for its activity. The unknown protein has to be located on the outside of the parasitophorous vacuolar membrane in order to pull proteins through.

Other experiments found that human HSP70 associates very strongly with the outer surface of the parasitophorous vacuolar membrane. This was interesting, because human HFP70 fulfils all the other identified criteria (i.e. it requires hydrolysed ATP, is present in non-infected cells and is trypsin-sensitive).

Recombinant HSP70 can be generated either in a wild type or a dominant-negative form. This was pipetted into the translocation mixture; human HSP70 was added in the dominant-negative form. It was found that if dominant-negative HSP70 is added on top of red blood cell cytosol, there is a 75% reduction in protein translocation to the host cell. It was concluded that HSP70 is definitely involved in the process, and there is a search for the other molecules that help it to carry out its function (e.g. HSP40 or nuclear-type exchange factors).

Further work was done by lysing red blood cells under certain conditions, resealing them and getting them to go back. While they are going back, it is possible to put other things in. In the experiment, dominant-negative HSP70 was inserted. The cells were resealed and the parasites were allowed to infect them. Controls were also carried out. When wild type HSP70 is added, there is some drop in the growth of the parasite, probably because the endogenous chaperone system is unbalanced. When dominant-negative HSP70 is added, there is a 75% drop in translocation.

This shows that HSP70 is not only involved in protein translocation, but also in parasite survival. It is not known whether there is an effect on the host cells (e.g. whether it stops the host cell being able to be invaded

by parasites), or whether the parasites need it directly. Further work is ongoing to establish this.

In summary, human protein is required for host cell modification and thus parasite survival. Inhibiting human protein has already been used in other contexts. For example, cancer cells are comparable to a parasite in that they are fast replicating, have an extremely high metabolism, and are dependent on a protein that normally replicating cells are not. Cancer cells have been inhibited using, among other things, inhibitors of molecular chaperones.

Even more importantly, if human proteins are inhibited, the parasite has no way to get around this. The parasite cannot change the composition of the human protein within the erythrocyte. This approach is thus not likely to be subject to parasite-driven resistance.

The parasite exports about 400 proteins into the host cell, but the function of most of these proteins is not known. It is suggested that they are likely to interact with human proteins, otherwise there is no reason for them to be present. It is thought that there is close interaction between parasite-exported proteins and human proteins, which is likely to be essential for the growth of the parasite. It is thus likely that there are other human targets to be discovered, and this is being investigated.

Dr Przyborski encouraged African delegates to explore the opportunities offered by the *Deutsche Forschungsgemeinschaft* (German Research Foundation) programme known as the German-African Cooperation Projects in Infectiology, which is designed to foster collaboration between German and African countries. The funding pays for one PhD student per group as well as consumables, staff travel and exchange between countries, and investment in infrastructure in Africa. The programme started in 2010, and 20 African countries have thus far been involved. The next round of pre-proposals opens in June and runs until November 2018.

Polymorphisms in HIV-1 *NEF* and *TAT* Associated with Endothelial Dysfunction (Ms Genevieve Mezoh, University of the Witwatersrand, South Africa)

Sub-Saharan Africa has a high prevalence of HIV. Africa accounts for about 70% of people living with HIV worldwide, and the rate of infection is growing the fastest in Africa.

Ms Mezoh posed the question of whether HIV is treatable, or curable. HIV is known to be transmitted from human to human. The virus is treatable using ARVs, but there is growing resistance among HIV-infected patients to taking the drug. Several strategies have been proposed with regard to an HIV cure, some of them being bogus:

- Traditional healers claim to be able to cure HIV in Africa. This affects the way in which the African population perceives information about HIV infection. This could perhaps account in part for the growing HIV prevalence in Africa.
- Myths abound, for example, that having sex with a virgin will cure HIV. People need to be sensitised about how HIV is spread, and the fact that there is no cure available yet.
- HIV vaccines have been proposed as a functional cure, but it will need to be established how efficient the vaccines are and what percentage immunity they will offer.
- It has been suggested that ultimately, humankind may exist in harmony with HIV.

Since the advent of ARVs, HIV-infected people are living much longer, and there has been a decrease in the incidence of opportunistic diseases. CVD have now emerged as an important cause of death among the HIV-infected population. Several studies have been released showing that the prevalence of CVD is higher in the HIV-infected population, but the pathophysiological process involved is not known.

Several studies have been conducted in the West looking at the prevalence of CVD in the HIV-infected population, but the emergence of CVD in the HIV-infected population in Africa has not been studied. There has been a proposal to investigate HIV and the risk of CVD in sub-Saharan Africa through the Ndlovu cohort study.

A healthy endothelium is the first line of defence against a pathogenic invasion because it is in contact with circulating pathogens. Ms Mezoh was investigating the hypothesis that unique polymorphisms observed in the HIV-1 *nef* and *tat* sequences may be associated with endothelial dysfunction in the HIV-infected population. The aim of the study was to assess endothelial dysfunction in the black South African HIV-infected population by measuring levels of blood markers of endothelial dysfunction and inflammation, and to assess the association of *nef* and *tat* gene variants with endothelial dysfunction.

For each biomarker, subjects were divided into two groups based on the median value for the biomarker. The frequency of each variant in these two groups was compared using a contingency table and two-tailed Fisher's exact test. Biomarker levels of the subjects possessing the identified variant were compared using a student's t test.

The study reached the following conclusions:

- There is evidence of endothelial dysfunction in the black South African HIV-infected population.
- The study found several mutations in HIV *nef* and *tat* associated with markers of endothelial dysfunction, confirming a role for these viral proteins in impacting endothelial function.
- Targeting HIV *nef* and *tat* is a potential therapeutic strategy to circumvent the development of CVD in HIV-infected patients.

Discussion

Comment (Dr Jude Przyborski): Stronger association might be found if patients with multiple genotypes were excluded.

Response (Ms Genevieve Mezoh): Because she was looking at the highest measurements it would not be possible to exclude patients with more than one variant.

Comment (Prof Anna Kramvis): The title of this session was The Big Four: HIV, TB, Malaria, HCV. It should have been 'The Big Five' and included hepatitis B, which needs to be included in surveillance because hepatitis B virus (HBV) is responsible for 90% of hepatitis in Africa, and there are 1.4 million deaths annually from HBV worldwide, which is equivalent to the death rate from HIV.

WRAP-UP, VOTE OF THANKS AND CLOSING OF THE SCIENTIFIC CONFERENCE

Prof Stefan Kaufmann, Director, Max Planck Institute for Infection Biology, Berlin, Germany

The first joint conference on infectious diseases between Leopoldina and ASSAf was held in October 2016 at the Max Planck Institute for Infection Biology in Germany. That meeting was a great success, but the present conference has been even more successful. Scientific information was shared, new friends were made, potential collaborators were identified, and researchers found ideas for new projects.

Prof Stefan Kaufmann conveyed the thanks of Prof Jörg Hacker, President of the Leopoldina, who had only been able to attend on the first day. It was particularly rewarding that Prof Hacker and Prof Jansen, President of ASSAf, had extended the MoU on cooperation between Leopoldina and ASSAf for another five years.

The conference included sessions on comorbidities, AMR, diagnostics, vaccines, drugs, patient-centred activities, management surveillance and response, and guidelines for the future.

Prof Kaufmann commended the title of the conference (Surveillance and Response to Infectious Diseases and Comorbidities: An African and German Perspective) for several reasons:

- Response is important from the WHO perspective.
- Comorbidities of infectious diseases with non-infectious diseases are becoming more frequent.
- The title places 'African' first before 'German', which reflects a meeting of the partners on an equal level.

Another positive aspect of the conference was that there was almost equal participation of men and women.

For future conferences on this topic between the partners, Prof Kaufmann would like to see the term 'integrated response' appear in the title.

Prof Kaufmann was glad to see initiatives in bioinformatics as a field for the future. His dream for the future was to have a disease epidemiology forecast. The response to the listeria outbreak in South Africa, for example, could have been considerably shortened through an information system that localised the occurrence of the disease and showed the supermarkets where the disease victims had bought their food. Attention would need to be paid to attract information technologists to develop these areas.

Prof Kaufmann thanked everyone responsible for organising the conference.

Mr Christian Acemah, Executive Secretary, Uganda National Academy of Sciences, Uganda

Mr Acemah commended the excellent balance at the conference between the German and African perspectives. He thanked everyone responsible for organising the conference. Unfortunately the scientific coordinator of UNAS, Prof David Serwadda, was not able to attend.

Prof Quarraisha Abdool Karim, Associate Scientific Director, CAPRISA, South Africa

Prof Karim echoed the thanks of the previous speakers to all those responsible for the partnership. Planning for the conference began in Berlin in 2016. There was a discussion of who to include, and particular interest in involving young investigators. The scope could have been limited to the partnership between Germany and South Africa, but it was decided to use the opportunity to try to make this a pan-African effort, and to widen the scope of German research institutions beyond Max Planck.

Senior scientists have a lot to offer at a conference such as this. They have been magnanimous in sharing their life-long experience and wisdom related to their successes and failures, projects, institution-building, programmes and peer collaboration.

The conference involved a good mix of senior scientists, mid-level researchers and junior investigators (or rising stars). Africa is unique in that 65% of the population of the continent is under the age of 35 years. In Africa, young people are not the future, but the present. The present generation of young people are growing up with technology and a worldview related to access to information that was previously inconceivable, and

which eliminates many of the disparities between the global North and South, and between industrialised and non-industrialised nations. Young investigators must not be underestimated; they have much to teach the older generation, and the older generation as much to share with them, in a synergistic relationship.

In the 21st century, there are possibilities of altering trajectories with respect to allowing people to live longer, healthier and more meaningful lives. Science is important for social and economic transformation. What humanity has already achieved is only the beginning. When men and women contribute equally, and when we can join forces globally, the possibilities on Earth and beyond are tremendous and are limited only by our imagination.

Surveillance

The Surveillance session did not take place the previous evening, so Prof Karim shared some ideas on this topic.

Epidemics tend to have a two-year delay before they are identified as an epidemic, whether category 1 or 2. Once the epidemic has been identified, there is media attention, huge investment and some level of public hysteria, but once the epidemic is over, amnesia sets in.

Those working with infectious diseases and disaster management need to be smarter during the inter-epidemic period to build on the momentum created by the epidemic. During the inter-epidemic period, we need to nurture relationships with the media and increase awareness among the general public. Popularisation of knowledge is important and does not receive enough attention, especially in Africa.

Epidemics start in communities, and communities are the first to know about the disease. They need to be informed how to respond when they observe common patterns and death. More advocacy efforts are needed at community level, linked to media efforts, whole government accountability, and more rapid responses. Good-quality, consolidated data and communication are important. There are many lessons to be learnt from each epidemic that can be applied to subsequent epidemics in order to develop a critical mass of individuals with knowledge, expertise and ability for rapid responses.

Prof Karim thanked the leaders and organisers responsible for convening the conference, the speakers and the participants. She acknowledged the sponsors: ASSAf, Leopoldina, ICSU ROA and the South African DST. Prof Karim invited participants to email suggestions for the evolving partnership.

APPENDIX A: LIST OF ACRONYMS

3TC	Lamivudine
ABR	Antibiotic resistance
AiBST	African Institute of Biomedical Sciences and Technology
AIDS	Acquired immune deficiency syndrome
AMR	Antimicrobial resistance
ARC	Agricultural Research Council
ARV	Antiretroviral
ASSAf	Academy of Science of South Africa
ATP	Adenosine triphosphate
AZT	Azidothymidine
BCG	Bacillus Calmette-Guérin vaccine
Bdq	Bedaquiline
BSL	Biosafety level
BTZ043	Benzothiazinone
BV	Bacterial vaginosis
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CARE	Collective Action and the Risk Ecosystem
CDC	Centre for Disease Control and Prevention
CDDEP	Centre for Disease Dynamics, Economics and Policy
CIDRZ	Centre for Infectious Disease Research in Zambia
CLSM	Confocal laser scanning microscopy
CSIR	Council for Scientific and Industrial Research
CVD	Cardiovascular disease
CYP	Cytochrome P450
DAAD	German Academic Exchange Service
DDS	Drug delivery system
DFID	Department for International Development
DHN	Dihydroxy naphthalene

DKA	Diabetic ketoacidosis
DIm	Delamani
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DoH	Department of Health
DRC	Democratic Republic of Congo
DST	Department of Science and Technology
EDCTP	European and Developing Countries Clinical Trials Partnership
EDIS	Extended Dose Interval Study
EFV	Efavirenz
EID	Early Infant Diagnostic
ErC	Erinacine C
EU	European Union
FAO	Food and Agriculture Organisation
FTC	Emtricitabine
G77	Group of 77 at the UN, a coalition of developing nations
GARD	Global Alliance Against Chronic Respiratory Diseases
GARP	Global Antibiotic Resistance Partnership
GDP	Gross domestic product
GIS	Geographic information system
GLASS	Global Antimicrobial Resistance Surveillance System
GOARN	Global Outbreak Alert and Response Network
HCV	Hepatitis C virus
HDSS	Health Research Centre (Manhiça, Mozambique)
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
HPV	Human papillomavirus
HSV	Herpes simplex virus
ICSU	International Council for Science
ICSU ROA	ICSU Regional Office for Africa

IDI	Infectious Diseases Institute, Uganda
IFS	International Foundation for Science
IMAI	Integrated Management of Adolescent and Adult Illness
IMCI	Integrated Management of Childhood Illness
INGSA-Africa	International Network for Governmental Science Advice – Africa
INH	Isoniazid
IPC	Infection prevention and control
LC	Liquid chromatography
LC-HRMS	Liquid chromatography-high resolution mass spectrometry
LMICs	Low and middle-income countries
Lzd	Linezolid
MBC	Minimum bactericidal concentration
MD PhD	Doctorate of Medicine and of Philosophy
MDR-TB	Multiple drug-resistant TB
MERS	Middle East respiratory syndrome
MIC	Minimum inhibitory concentration
MoU	Memorandum of understanding
MS	Mass spectrometry
Mtb	Mycobacterium tuberculosis
NAP	National action plan
NCD	Non-communicable disease
NGO	Non-government organisation
NIH	National Institutes of Health
NMIMR	Noguchi Memorial Institute for Medical Research
NMR	Nuclear magnetic resonance spectroscopy
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NP	Nanoparticle
NRF	National Research Foundation
NSP	National Strategic Plan
NVP	Nevirapine

NWU	North-West University
OECD	Organisation for Economic Co-operation and Development
OiE	World Organisation for Animal Health
OPSCC	Oropharyngeal squamous cell carcinoma
OSCC	Oral squamous cell carcinoma
PCR	Polymerase chain reaction
PEN	Package of Essential Non-communicable Intervention Tools
PEPFAR	US President's Emergency Plan for AIDS Relief
PfEMP1	Plasmodium falciparum erythrocyte membrane protein 1
PhD	Doctor of philosophy
PID	Pelvic inflammatory disease
PLGA	Polylactic-co-glycolic acid
PMTCT	Prevention-of-mother-to-child-transmission
R&D	Research and development
RIF	Rifampicin
RNA	Ribonucleic acid
RR-TB	Rifampicin-resistant TB
RISLNET	Regional Integrated Surveillance and Laboratory Network
RVF	Rift Valley fever
SAMRC	South African Medical Research Council
SARChI	South African Research Chairs Initiative
SARS	Severe acute respiratory syndrome
SATVI	South African Tuberculosis Vaccine Initiative
STI	Sexually transmitted infection
TB	Tuberculosis
TDF	Tenofovir disoproxil fumarate
TEM	Transmission electron microscopy
TLC	Thin layer chromatography
TWAS	The World Academy of Sciences

UK	United Kingdom
UNAS	Uganda National Academy of Sciences
USA/US	United States of America
UV	Ultraviolet
VHF	Viral haemorrhagic fever
VPM	Vakzine Projekt Management
WHO	World Health Organisation
WHO-AFRO	WHO Regional Office for Africa
WNV	West Nile virus
XDR-TB	Extensively drug-resistant tuberculosis
ZP	Zeta potential

APPENDIX B: LIST OF PARTICIPANTS

NAME	SURNAME	TITLE	INSTITUTION/COMPANY
Quarraisha	Abdool Karim	Prof	CAPRISA
Salim	Abdool Karim	Prof	CAPRISA
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Annah Margret	Biira	Dr	Makerere University
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Siyavuya	Bulani	Dr	Academy of Science of South Africa
Madichaba	Chelopo	Ms	North-West University
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Dina	Coertzen	Dr	University of Pretoria
Halima	Dawood	Dr	Greys Hospital
Nokwanda	Depargo	Mrs	CAPRISA
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Dorcas Oladayo	Fatoba	Mrs	University of KwaZulu-Natal
Lindiwe	Faye	Ms	Nelson Mandela Academic Clinical Research Unit
Glenda	Gray	Prof	South African Medical Research Council
Sherika	Hanley	Dr	CAPRISA
Rose	Hayeshi	Prof	North-West University

NAME	SURNAME	TITLE	INSTITUTION/COMPANY
Jörg	Hecker	Prof	German National Academy of Sciences Leopoldina
Norbert	Heinrich	Dr	University Hospital LMU Munich
Eva	Herker	Dr	Leibniz Institute for Experimental Virology
Shevin	Jacob	Dr	Infectious Diseases Institute/Liverpool School of Tropical Medicine
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Alison	Lubisi	Dr	Agricultural Research Council
Andriy	Luzhetskyy	Prof	Helmholtz Institute for Pharmaceutical Research
Tebogo	Mabotha	Dr	Academy of Science of South Africa
Steven	Manzi	Mr	Vaal University of Technology
Stanley	Maphosa	Mr	Academy of Science of South Africa

NAME	SURNAME	TITLE	INSTITUTION/COMPANY
Tebogo	Maphosa	Mr	Kaboentle Centre for Life
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Jan	Nilsen	Dr	German National Academy of Sciences Leopoldina
Oladoyin	Odubanjo	Dr	Nigerian Academy of Sciences
Olatunde	Olayanju	Dr	University of Cape Town
Charles	Osunla	Mr	Fort Hare University
Sean	Patrick	Dr	University of Pretoria
Jude	Przyborski	Dr	Heidelberg University Hospital
Caroline	Pule	Ms	Stellenbosch University
Kristina	Roesel	Dr	International Livestock Research Institute

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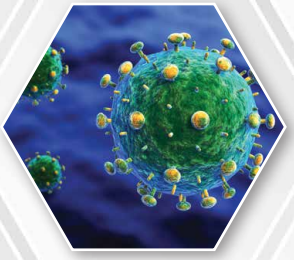
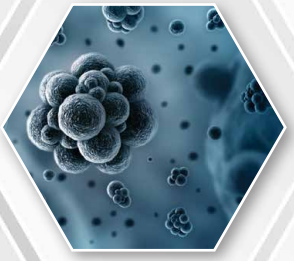
Renate	Venier	Ms	Academy of Science of South Africa
Marietjie	Venter	Prof	University of Pretoria
Delfino	Vubil	Mr	Manhica Health Research Centre
Henriette	Wagener	Ms	Academy of Science of South Africa
Kathrin	Wittstein	Dr	Helmholtz Centre for Infection Research
Charles	Wiysonge	Prof	South African Medical Research Council





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